THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII JANUARY, 1942 NUMBER 1

SCLEROSING HEMANGIOMAS OF THE CENTRAL NERVOUS SYSTEM * PROGRESSIVE TISSUE CHANGES IN HEMANGIOBLASTOMAS OF THE BRAIN AND IN SO-CALLED ANGIOBLASTIC MENINGIOMAS

ORVILLE T. BAILEY, M.D., and RICHARD FORD, M.D.

(From the Departments of Pathology of the Harvard Medical School and of the Peter Bent Brigham Hospital, and from the Fourth Surgical Service of the Boston City Hospital, Boston, Mass.)

A comparatively small but pathologically interesting and clinically important group of intracranial tumors are of blood-vessel origin. Blood vessels are structures common to the tissue patterns of the brain and of organs outside the central nervous system. For this reason, tumors of blood-vessel origin in the brain and its covering might be expected to resemble in most respects those arising in the blood vessels of other organs. However, the fat, pigment and neuroglia content of intracranial hemangiomas have suggested that there are important differences between the two groups. This discrepancy has led to speculation as to the possibility of variations in the cells of origin; comparatively little attention has been paid to the histological sequences which take place during the growth of hemangiomas, regardless of the site at which they originate.

This paper presents a study of the progressive tissue changes in hemangiomas of the central nervous system and a comparison of these tissue changes with those which take place in hemangiomas of the skin and other organs. The difficulties in identifying the histological sequences concerned in the growth of intracranial tumors of blood-vessel origin are increased by their comparative rarity and the consequent difficulty in securing specimens which represent successive stages of the lesions. Furthermore, material secured at operation preserves few of the relationships of the tumors to surrounding structures. It is much easier to secure a satisfactory series of cutaneous hemangiomas. Our studies, then, have been made on three sorts of hemangiomas: a series of peripheral tumors, mostly of the skin; a group of hemangioblastomas of the brain; a group of angioblastic meningiomas. Study

* Presented at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 11, 1941.

Received for publication, June 2, 1941.

of these three has shown that, in each, histological sequences take place which are identical when account is taken of the differences in the surrounding tissues.

Sclerosing Hemangiomas of the Skin and Other Extracranial Structures

In certain hemangiomas of the skin and occasionally in those of other extracranial organs, a process of sclerosis takes place. In connection with this, progressive tissue changes occur which result in so marked an alteration in the morphology of the hemangiomas that their true nature is often overlooked.

Wolbach,[1] in 1913, published a brief discussion of a process of sclerosis in cutaneous hemangiomas. This note described clearly the tissue sequences involved and their consequences as illustrated by two examples. In the 28 years since that publication appeared, he has collected a large series of sclerosing hemangiomas which confirms and extends the original description. A detailed report on this material has recently been prepared in his laboratory.[2] Since the alterations which occur during the process of sclerosis in cutaneous hemangiomas are directly comparable to the progressive tissue changes in tumors of blood vessels arising in the brain and its coverings, a brief review will be given here.

Cutaneous hemangiomas consist of blood vessels, among which there is a stroma of collagenous tissue which acts as a supporting framework for the neoplasm. When sclerosis occurs, the stroma increases in amount, either diffusely or in foci. It has so far been impossible to detect what starts the process of sclerosis but, once started, the process leads to a clearly defined sequence of cellular changes. The increase in connective tissue results in the occlusion of vessels, either directly or through later contraction. The red blood cells thus trapped disintegrate and their degeneration products are taken up by phagocytes. In this way, the hemangiomas come to contain hemosiderin-filled cells, at times in sufficient number to give a brown color to the tissue. The phagocytic cell nuclei are identical with those of the endothelium in the blood vessels of the tumors. Occasionally some of the pigment is taken up by endothelial cells while still in their usual location about the vascular lumina. For these reasons, it appears that the phagocytes are of endothelial-cell origin.

Some endothelial cells of the tumor take up fat—evidently fat of normal character in the circulating blood (that is, nutritional fat). It is possible that some of the fat is derived from the disintegrating red blood cells. However, the tumors in which the fat is most abun-

dant seldom contain large quantities of hemosiderin and those brown with hemosiderin usually contain small amounts of fat. The lipoid content of the hemangiomas richest in this material seems too great to be accounted for by the number of red blood cells which might have disintegrated. Therefore, it is likely that most, if not all, of the fat is derived from the blood serum. As vessels become obliterated, the relation of the fat-filled cells to their walls is obscured. In this way, large masses of cells filled with hemosiderin and lipoid material accumulate without apparent connection with the vascular lumina. The groups of fat cells give a yellow color to the parts of the tumors in which they are numerous.

In most of the sclerosing hemangiomas, giant cells make their appearance. The morphology of the nuclei and their relationship to other cells in the surrounding tissue suggest that the giant cells are of endothelial origin. In some specimens, the giant cells are few but in occasional examples they are so numerous as to be very conspicuous in the general histological picture.

The degree of sclerosis varies from specimen to specimen. It may be absent, slight, or marked. The other tissue changes will then vary accordingly. Furthermore, the proportion of fat, hemosiderin and of giant cell formation may vary greatly in different examples. If only a few specimens are studied, especially when they happen to represent different extremes of the process, a common origin may be overlooked. For this reason, some sclerosing hemangiomas have been called giant cell xanthomas, and some histiocytomas. Many other names have also been applied to tumors of this group; for instance, it appears that the giant cell endothelioma of tendon sheaths is also a sclerosing hemangioma. These tumors have been regarded, from time to time, as localized manifestations of a general disturbance in lipoid metabolism. Localized subcutaneous deposits are found in patients with such disturbances of lipoid metabolism as Schüller-Christian's disease. Careful histological study makes it certain that sclerosing hemangiomas are lesions of entirely different character than such localized lipoidal deposits. Complete postmortem examination in some of the patients with sclerosing hemangiomas has failed to show any evidence of a generalized disturbance of lipoid metabolism.

General Considerations in Regard to Blood-Vessel Tumors of the Brain and Meninges

The recognition of the histological sequences just discussed has made it possible to include among the peripheral hemangiomas a considerable number of tumors previously regarded as different in histogenesis.

Because of this, it seemed worth while to examine a series of intracranial tumors originating in blood vessels to determine whether similar sequences might explain the supposed differences between these tumors and hemangiomas elsewhere.

Cushing and Percival Bailey,[3] in their monograph dealing with blood-vessel tumors of the brain, divided these lesions into angiomatous malformations and true neoplasms, *i.e.*, hemangioblastomas. A group of tumors originating in the meninges was described by Percival Bailey, Cushing and Eisenhardt [4] under the name *angioblastic meningioma*. The angiomatous malformations have not been considered in this study since they lack the characteristics of progressive tissue growth. There was available for study a series of 20 intracranial tumors of blood-vessel origin, partly from the service of Dr. Harvey Cushing and partly from that of Dr. Elliott C. Cutler. Of these, 16 were hemangioblastomas, 15 located in the cerebellum and 1 in the spinal cord. Four were of the type called angioblastic meningiomas. All tumors in which the material was fragmentary or poorly fixed were excluded from the series. Fixation was in Zenker's fluid and in a 4 per cent solution of formaldehyde. The following histological methods were used: hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue connective tissue stain, Foot-Bielschowsky's method for reticulum, Laidlaw's method for reticulum and Mallory's method for iron. When unembedded formaldehyde-fixed material was available, Scharlach R and osmic acid were used for the demonstration of fat.

It will be noted that the terms *hemangioma* and *hemangioblastoma* have both been used in this paper. *Hemangioma* is the one most often employed by general pathologists for blood-vessel tumors of the skin and other viscera. *Hemangioblastoma* is used especially by neuropathologists to apply to blood-vessel tumors of the brain, largely as the result of the publications of Dr. Cushing's clinic. The latter term has the slight advantage of emphasizing the fact that the lesions are neoplasms and not malformations. The two terms are used interchangeably in this paper, and no special significance is intended when one is chosen rather than the other.

Hemangioblastomas of the Brain

The clinical and gross anatomical features of hemangioblastomas of the brain are well known. They are summarized in the monograph of Cushing and Percival Bailey.[3] As this publication contains an extensive review of the literature to 1928, the survey has not been repeated here. Since 1928 there have been a number of reports dealing with

the subject from various points of view but not adding material relevant to the present discussion. A useful and well illustrated paper of the group is that of Sargent and Greenfield.[5]

These tumors are much more common in the cerebellum than in other parts of the brain. Fifteen tumors in the present series were located in the cerebellum; the sixteenth was a histologically similar lesion of the spinal cord. The question of cerebral hemangioblastomas is discussed in the part of this paper devoted to angioblastic meningiomas.

Those hemangioblastomas situated within the brain substance are occasionally solid but more often form a mural nodule of a cyst. Histologically, some of them are composed of large cavernous blood spaces and others are made up of vessels of capillary size (Fig. 1). In both instances, collagenous connective tissue extends among the blood vessels so as to form a supporting stroma. The resemblance of such blood-vessel tumors to those of the skin, liver and other extracranial organs is obvious.

There are, however, other hemangioblastomas in which the vessels are separated from one another by cellular areas (Fig. 2). The cells in these regions are supported by a delicate reticulum (Fig. 3). In accordance with the view of Mallory and Parker,[6] supported by the experimental work of Wolbach,[7] we have regarded the reticulum as a stroma of fine collagen fibers. As shown in Figure 3, the silver impregnation renders more conspicuous the occasional capillaries in the cellular areas. The methods for reticulum bring out very clearly the connective tissue fibers beneath the endothelial cells. In sections prepared with other stains, the capillaries may be indistinct because they are empty and collapsed. Some of the cells in the intervascular tissue contain fat and are spoken of as "xanthoma" or "pseudoxanthoma" cells. These features and a variable number of neuroglia cells in some portions of the tumors have led neuropathologists to set these hemangioblastomas apart from other tumors of blood-vessel origin. Phagocytes containing hemosiderin, which occur in these hemangioblastomas, have attracted less attention. On the other hand, these features, except for the neuroglia, are exactly those which make their appearance as hemangiomas outside the brain undergo sclerosis.

Presentation of a representative instance of cystic hemangioblastoma of the cerebellum will assist in the discussion of the progressive tissue changes in these tumors.

Case 1

Headache, visual disturbances, personality changes. Cerebellar exploration disclosing cyst with mural nodule. Fixation of wall without removal of nodule. Gradual recurrence of symptoms after 11 years. Re-exploration 13 years after first

operation with complete extirpation of hemangioblastoma (sclerosing hemangioma) of cerebellum. Death from postoperative hemorrhage.

Mr. C. D. was a laborer, 39 years old, who was admitted to the hospital because of headaches. The onset of the patient's illness 5 years previously was characterized by attacks lasting about 1 minute in which "things would go blank," which occurred as he was getting up or lying down. After 3 months, these attacks were replaced by headaches, nausea and vomiting. The headaches increased in frequency and severity and later were accompanied by dizziness and tinnitus. The patient felt that he was becoming irritable and more easily upset than previously. Disturbances in vision made their appearance 2 months before admission here. He had been admitted to another hospital 1 month before coming to this hospital, after a period of unconsciousness lasting 12 hours. There a ventricular puncture was performed, after which the patient left against advice.

At the time of admission to the Peter Bent Brigham Hospital, the general physical examination revealed no abnormal findings. On neurological examination, there was bilateral papilledema (right eye, 3 diopters; left eye, 4 diopters) and slight nystagmus to the right. During a severe headache the Romberg sign, previously negative, became positive, the patient falling to the right. Coincident with this, marked ataxia appeared in both upper extremities and the patient leaned to the right when he walked. The nystagmus was unchanged during the attack of headache. Roentgenograms showed no changes to assist in localization of the tumor.

A cerebellar exploration was carried out. When the cerebellum was exposed, the thin wall of a cyst was brought into view. The cyst originated in the right cerebellar hemisphere near the midline. About 15 cc. of straw-colored fluid were obtained when the cyst was needled. The surface of the cerebellum was highly vascular. An incision was made in the cyst wall. After thorough fixation of the cyst wall with Zenker's fluid, the lining puckered everywhere except at a point on the lower surface of the cyst where a reddish nodule of tumor came into view. It did not seem wise to prolong the operation sufficiently to remove the nodule.

Postoperative recovery was rapid, and the patient remained symptom-free for 11 years. He then had one severe attack of headache. The only residuum of this attack was a tendency to stagger to the right. Two years later, headache again appeared at intervals and at this time was accompanied by nausea. The patient was readmitted to the hospital after 10 days of severe occipital headaches. Examination at that time elicited tenderness in the suboccipital region; the neck was somewhat rigid. Partial anosmia had made its appearance. The visual acuity was 20/200 in both eyes. The right optic disk showed slight atrophic changes and papilledema of 1 diopter. There was papilledema of 2 diopters on the left. Nystagmus to the right was present on two occasions. The patient had a tendency to stagger to the right, and fell in the same direction when the Romberg test was tried. The pharyngeal reflex was absent. Adiadokokinesia was brought out by the usual tests. The abdominal reflexes were absent on the right; all deep reflexes were hypo-active. The only abnormality in the roentgenograms was the operative defect in the skull.

Suboccipital exploration was undertaken and the field of the former procedure again exposed. After removal of the spine and lamella of the atlas, a tumor the size of a golf ball was brought into view in the right cerebellar hemisphere. The tumor was partly cystic, the cavity containing yellow fluid which immediately clotted. The entire tumor was enucleated.

The postoperative course was stormy. Because of slow pulse and respirations, the bone flap was re-elevated on the night after operation. The patient remained in an unsatisfactory condition and death occurred on the sixth hospital day.

Autopsy was limited to examination of the brain. There was a

large blood clot in the cavity from which the tumor was removed and over the cisterna magna. No residual tumor could be found.

The material removed at operation was promptly fixed and proved excellent for study. It consisted of a nodule of tumor weighing 12 gm. and measuring 3.2 by 1.8 by 2 cm. The specimen was soft in consistency. The surface was in part covered by a thin capsule while elsewhere it was irregular. Some cerebellar tissue was attached to one border. The cut surface showed pinkish gray tissue with many small yellow areas.

Microscopical examination showed the tumor to be of vascular origin. It was composed of blood vessels ranging in size from very large irregular blood spaces to minute capillaries. The larger blood vessels contained moderate numbers of blood cells. They were lined by endothelial cells, usually in one layer, though here and there small clusters of these cells could be found. The endothelium of a very few vessels was surrounded by layers of smooth muscle. In nearly all vessels, however, only connective tissue lay outside the endothelium. This varied in amount from a few collagen fibers to wide collars of partially hyalinized collagenous tissue. The capillaries sometimes contained blood but were more often empty. Their walls consisted of a layer of endothelium with collagen fibers beneath, often only a single fiber outlining the endothelial-lined space.

The intervascular tissue consisted of closely-packed cells in a delicate reticular stroma. Coarse collagen fibers were found only in occasional areas. The tumor cells between blood vessels had oval nuclei with a distinct nuclear membrane and well dispersed chromatin; mitotic figures were rare. The cytoplasm was acidophilic. The general morphological characteristics of the tumor cells in the intervascular tissue were identical with those of the endothelium lining the blood vessels. In some areas (corresponding to the regions which appeared yellow grossly), the cytoplasm of the tumor cells contained medium-sized droplets of fat, which stained orange-red with Scharlach R and black with osmic acid. Cells containing lipoid were confined almost entirely to the intervascular tissues but endothelial cells in the lining of the vessels contained fat droplets in rare instances. There were phagocytic cells filled with hemosiderin scattered in foci through the tumor. These cells were clustered together in the areas where collagen was most abundant.

In portions of cerebellum attached to the specimen, the astrocytic feltwork was increased in amount and in density. Astrocytes were included in the margin of the tumor and a few of them extended for a distance into it. The neuroglia content of this tumor, however, was not so great as that of some other neoplasms of the series.

PROGRESSIVE TISSUE CHANGES IN HEMANGIOBLASTOMAS OF THE BRAIN

It is clear that such tumors as that found in case 1 are of blood-vessel origin. The evidence for this lies not only in the number of atypical larger vessels in the neoplasm but especially in the presence of minute, poorly formed capillaries even in the most cellular portions and in the resemblance of the cells in the intervascular portions to endothelial cells. In order to explain the differences between the histology of these tumors and that of the more familiar tumors of blood-vessel origin (Fig. 1) it is necessary to find either peculiarities of the cells from which neoplasms originate or progressive sequences of tissue behavior by means of which the morphology becomes changed.

There are two important indications that histological sequences are involved rather than differences in the cells of origin. The hemangioblastomas of the cerebellum may be arranged in a series, placing at one extreme the tumors composed of fairly typical blood vessels with a collagenous stroma and at the other, tumors such as that described in case 1. Between the two extremes, a variety of intermediate stages are found. The cellular areas with fat-laden cells, the neuroglia content and the hemosiderin pigmentation may be absent, slight or marked. The likelihood of these intermediate stages being due to progressive tissue changes is enhanced by a second fact—that similar accumulations of phagocytes filled with hemosiderin and lipoid material are known to result from a process of sclerosis in hemangiomas occurring in organs other than the brain.

The sequences of sclerosis are very similar wherever the blood-vessel tumor takes origin. An overgrowth of stroma results in occlusion of some of the blood vessels. Disintegration products of blood, thus trapped, appear in phagocytes. Giant cells are occasionally found in the sclerosing hemangioblastomas of the brain, but they did not form a conspicuous part of the histological picture in any of the tumors in this series.

As already pointed out, the presence of phagocytes containing lipoid has been regarded as an important feature of the histology of hemangioblastomas of the brain. A few of the endothelial cells of still patent capillaries pick up fat, at first in small droplets and later in amounts sufficient to occupy most of the cytoplasm. A large number of the lipoid-laden phagocytes lie between the vessels. The phagocytes appear to be endothelial in origin. The evidence points to the blood stream as the source of the fat because of its presence in occasional endothelial cells lining patent vessels and because there is no indication of a generalized disturbance of lipoid metabolism. It is difficult on purely morphological grounds to exclude the possibility that some of the fat is derived from degenerating myelin. Since the sequences in which the

lipoid phagocytes appear and their distribution are similar to those of morphologically identical cells in sclerosing hemangiomas outside the central nervous system, it would appear that the fat has a common origin in all locations. This makes it unlikely that any important part of the lipoid is derived from the degeneration products of myelin.

The presence of neuroglia seems at first difficult to explain on the basis of progressive tissue changes. This can be done, however, when the nature of the stroma is considered further. The stroma of peripheral hemangiomas is derived from the connective tissue about the lesion. It consists of collagen fibers and the cells which produce and maintain them. When the stroma increases in the process of sclerosis, the change is in quantity rather than in character of the stromal cells and their fibers. In the brain, the supporting tissue under normal conditions is of two kinds. One of these is collagenous connective tissue which is associated in distribution with the blood vessels of the brain. The other is the ectodermal stroma, that is, the network of astrocytes, which participates in the support of the nerve cells and their processes. Both mesodermal and ectodermal stroma are available to take part in the histological sequences of sclerosis when they occur in blood-vessel tumors of the central nervous system.

Most of the stroma of some hemangioblastomas of the brain is composed of collagenous connective tissue. When the neoplastic blood vessels infiltrate the surrounding brain substance, the nerve cells and their fibers are destroyed as far as infiltration extends. Some of the glial supporting tissue escapes, and, being a tissue with considerable capacity for growth, its cells proliferate. They then become incorporated in the hemangioblastoma as a part of its stroma. Since the astrocytes are usually confined to the outer zone of the tumors and are in all our cases most abundant there, the indications are that the neuroglia becomes available only when the tumor has extended into the adjacent brain.

Because there are several factors in the process of sclerosis which vary independently, the number of possible histological pictures in sclerosing hemangiomas of the brain is very large. Many superficially different histological pictures may result from what is essentially one process. It is possible to overlook the fundamental similarity of specimens representing different extremes unless intermediate specimens have been studied and the progressive tissue changes defined.

So-Called Angioblastic Meningiomas

In 1928, Percival Bailey, Cushing and Eisenhardt [4] described a group of meningeal tumors of blood-vessel origin which they called *angioblastic meningiomas*. In defining the term, they stated: "By the

term *angioblastic meningioma* we do not mean meningiomas that merely happen to be highly vascular in the sense that the term angioglioma is sometimes used for a highly vascular glioma. What we do mean is that a meningeal tumor which possesses the unmistakable naked-eye appearance of the usual and familiar type of one of these lesions may prove histologically to have the same architectural features and reticular network that is possessed by the hemangioblastomas which originate within the brain itself, more frequently, if not exclusively, in the cerebellum." In other words, the distinction between angioblastic meningiomas and hemangioblastomas is one of location only.

The study of the so-called angioblastic meningiomas in the present series has confirmed the statement that these tumors are of blood-vessel origin. It has indicated, furthermore, that the same progressive tissue changes are present in these tumors as in hemangioblastomas of the brain substance, skin and other organs. It would hardly be worth while to discuss separately in this paper two groups of tumors differing only in location if it were not for the fact that a number of publications have appeared in which either the similarity of hemangioblastomas of the brain and angioblastic meningiomas has been overlooked or an attempt has been made to relate angioblastic meningiomas to the meningeal tumors originating in arachnoid cells. The literature dealing with angioblastic meningiomas has recently been summarized by Courville and Abbott.[8]

The lesions of this type described in publications have been located almost entirely above the tentorium. However, some few cerebellar tumors can hardly be excluded from the group. Two of the tumors in the present series were located in the occipital lobe, the other two in the cerebellum. The cerebral tumors presented the characteristics of angioblastic meningiomas as described by Percival Bailey, Cushing and Eisenhardt [4] and elsewhere. We have chosen, however, for detailed report one of the cerebellar tumors with several atypical features. It arose from the meninges and pushed the cerebellum before it, infiltrating its structure only very slightly. Thus, although it behaved in respect to the brain tissue as typical angioblastic meningiomas do, it had no dural attachment. Since it had not been approached surgically, the relations to brain and meninges could be studied. The tumor has many features both of hemangioblastoma and of angioblastic meningioma. It may be regarded as a transitional form between the two categories, but it seems to us to strengthen the opinion that hemangioblastomas of the brain and angioblastic meningiomas are, from the pathological standpoint, one and the same, except in point of origin.

Case 2

Headaches, vomiting, visual disturbances and personality changes. Subtemporal decompression performed elsewhere. By ventriculography, poor filling of third ventricle with displacement of its posterior portion to the left. Death from bronchopneumonia. Sclerosing hemangioma of cerebellar meninges found at autopsy.

Mrs. M. LeB. was a French-Canadian mill-worker, 53 years old, who came to the hospital because of headache and visual difficulties. For the year preceding admission, she experienced headaches, which became very severe during the last 5 months of the interval. As the headaches increased in severity, they came to be associated with vomiting, not preceded by nausea. About 6 weeks before entering the hospital, she noticed blurring of vision in the right eye, followed by blindness in that eye 9 weeks later. Four weeks before entry, vision became blurred in the left eye. The patient's memory began to fail; there were visual and auditory hallucinations on several occasions. Two weeks before admission here, a subtemporal decompression had been done elsewhere with partial relief of symptoms. The patient was transferred to the Peter Bent Brigham Hospital for further study and treatment.

On arrival at this hospital, the only abnormalities noted on general physical examination were bulging in the area of subtemporal decompression and moderate cardiac enlargement. The neurological examination revealed the sense of smell to be somewhat diminished. There was no perception of light in the right eye. The right pupil did not react to accommodation or to light directly, though there was a reaction to light consensually. The palpebral fissure was narrowed and internal strabismus of the right eye was present. There was marked diminution of vision in the left eye and the pupil was dilated but not fixed. Papilledema was present to the extent of 3 diopters in the right eye and 1 diopter in the left eye. There was slight nystagmus to the right. Both corneal reflexes were sluggish and increased lacrimation was noted in both eyes. There was a partial left lower facial palsy. A definite dysarthria was combined with a tendency to loquaciousness. The patient responded to tests for adiadokokinesia slowly but accurately. Temperature sensation was absent over the entire left half of the body from crown to toe, as were tactile sensation and ability to discriminate pain and pressure in the same area. The pharyngeal reflex was absent. Tendon reflexes were active and equal; the right upper was the only abdominal reflex obtained. Stereoroentgenograms of the skull showed no definite localizing signs of intracranial tumor.

Ventriculography was carried out on the fourth hospital day. The ventriculograms showed definite dilatation of both lateral ventricles but no localized deformity. The third ventricle filled poorly; its posterior portion was displaced slightly to the left. The patient's postoperative course was stormy and death occurred on the 13th hospital day, further operation not being carried out.

Anatomical diagnoses: Sclerosing hemangioma of meninges; bronchopneumonia; bacteremia (*Staphylococcus aureus*); multiple abscesses of lungs; arteriosclerosis, generalized; multiple leiomyomata of uterus.

The findings relevant to the present discussion were limited to the brain. There were no hemangiomas in other organs.

The region of the subtemporal decompression was bulging. The dura was tense, the convolutions flattened and the sulci narrowed. There was a tumor 1 cm. deep and 2 cm. in length in the posterior portion of the right cerebellar lobe. This was attached to the leptomeninges and bulged above the adjacent brain tissue, but there was no dural attach-

ment. The tumor had a distinct yellowish color. Not only was the tumor itself highly vascular but the blood vessels of the meninges about it were increased in size and number.

On cross section the fresh tumor was predominantly orange in color. The relations of the neoplasm were best studied in large sections, which included a cross section of the entire tumor and adjacent portions of the cerebellum (Fig. 5). This showed that the tumor was situated superficially with its outer surface covered only by an attenuated arachnoid membrane. The neoplasm occupied largely a position corresponding to a greatly widened subarachnoid space. As a result, the underlying cerebellar folia were pushed aside and flattened while adjacent cerebellar folia were normal in size and shape. The tumor, then, appeared from its gross relationships to have arisen in the leptomeninges and to have caused its effects on the cerebellum by pressure. Closer inspection, however, showed that the superficial portions of some of the folia had been invaded for a short distance. Although the tumor was primarily a tumor of the leptomeninges, it extended through the pia for a short distance into the underlying cerebellum (Fig. 7). In this it resembled the meningiomas of arachnoid-cell origin which pass the pial barrier.[9, 10]

Microscopical Examination. The tumor was characterized by the presence of blood vessels separated by areas of closely packed cells. The vascular spaces varied in size from large, irregular vessels to minute capillaries. The vessels were lined with flattened endothelial cells in a single row, or with swollen, closely set endothelial cells in small masses. Some of these cells contained granules of hemosiderin, and in others there were droplets of lipoid material in the cytoplasm. The endothelial cells were surrounded in the larger blood spaces by a rather thin zone of collagenous tissue. Occasionally, the collagen bundles were swollen and separated by edema so that there were large, relatively acellular collars around the vessels. About the endothelium of the finer capillaries, single delicate strands of collagen blended with the reticulum of the intercapillary cell clumps (Fig. 11).

The tissue separating the blood vessels was composed of closely packed cells in a delicate reticulum. At intervals, however, minute empty capillaries could be outlined with reticulum stains (Fig. 11). The cells in the intervascular tissue were endothelial in type. The oval nuclei were surrounded by a distinct nuclear membrane and the moderate amount of chromatin was well dispersed. Mitotic figures were occasionally encountered but were not numerous. The cytoplasm of the tumor cells was acidophilic and in some cells contained granules of hemosiderin (Fig. 8). These pigment phagocytes were concentrated

in areas where collagen was abundant and tumor cells relatively few. In a large percentage of the cells, there were droplets of fat in the cytoplasm (Fig. 9). Usually these fat-filled cells were packed in large masses separated by bands of collagenous tissue from which delicate reticular fibers extended into the masses and formed a stromal network for the fat-filled cells. Occasional multinucleated giant cells were found in the intervascular tissue.

The free edge of the tumor was covered by an intact layer of arachnoid cells with rather dense collagen fibers beneath it. While the fat-filled cells were abundant throughout the tumor, they were especially numerous near the free edge of the neoplasm. Neuroglia cells were not present in this region. On its deep surface, the tumor invaded for a short distance the granular and molecular layers of the underlying cerebellar cortex. In the part of the tumor nearest the region of invasion, numerous fine capillaries, intercapillary endothelial cells, collagen fibers and astrocytes were mingled in haphazard fashion (Fig. 10). The astrocytes extended into the tumor for some distance toward the center. Occasionally, small groups of astrocytes surrounded capillaries and were attached to their walls by sucker feet. Only very close to the advancing edge were there any nerve cells in the tumor. The architecture of the cerebellar cortex immediately below the neoplasm was somewhat distorted from pressure but elsewhere it was normal (Figs. 5 and 7).

A Comparison of Angioblastic Meningiomas and Hemangioblastomas of the Brain

As pointed out earlier in the discussion, case 2 has features which are in part those of the tumors described as hemangioblastomas of the cerebellum and in part those of angioblastic meningiomas. Similarities to cerebellar hemangioblastomas are its location below the tentorium and its lack of dural attachment. The resemblance to angioblastic meningiomas is its origin from the meninges. The detailed histology of both varieties of tumors is identical, as pointed out by Percival Bailey, Cushing and Eisenhardt.[4] Case 2, therefore, seems important in studying the resemblances and differences of hemangioblastomas of the brain and angioblastic meningiomas.

The angioblastic meningiomas are solid tumors of meningeal origin and are by far more frequent above the tentorium than below. It has been suggested by some writers that all cerebellar tumors of vascular origin should be excluded from this group. However, the neoplasm in case 3 of the report by Percival Bailey, Cushing and Eisenhardt [4] probably originated in the cerebellar meninges, although at autopsy the

tumor was found in the occipital region as well as in the cerebellum. This statement is made because the initial symptoms were those associated with cerebellar involvement; symptoms of occipital lobe involvement did not appear until 2 years after the first operation. This and an example in the present series (in addition to case 2) indicate that angioblastic meningiomas may arise in the cerebellar meninges.

In contrast, the hemangioblastomas are rarely seen in the cerebrum. Cushing and Percival Bailey's [3] examples of this lesion were all cerebellar. Some of the reported hemangioblastomas of the cerebrum seem identical with those of the cerebellum. The hemangioblastomas may be solid or partly cystic but more often form a mural nodule of a cyst, which is elsewhere lined by a condensation of glia. For this reason, the tumor described by Barnard and Walshe [11] as a capillary hemangioma of the cerebrum seems certainly acceptable in the group of hemangioblastomas of the brain substance. There was a cyst in the right temporal lobe with a mural nodule identical in histology with the nodules in hemangioblastomatous cysts of the cerebellum. It is interesting to contrast this lesion with the tumor in case 2 of the present series. Barnard and Walshe's tumor was a cyst with a mural hemangioblastomatous nodule in the substance of the cerebrum when statistically a solid tumor of the cerebral meninges would be expected in that location. The lesion in case 2 of the present series was a solid tumor of the cerebellar meninges when statistically a cyst with a mural nodule would have been expected. It seems more logical to say that hemangioblastomas of the cerebrum are usually solid and attached to the meninges while those of the cerebellum usually form the mural nodule of cysts rather than to set these lesions apart as separate entities.

It has been pointed out that angioblastic meningiomas have an attachment to the dura.[4, 8, 12] The absence of dural attachment in the tumor of case 2 might be regarded as evidence that the tumor is not an angioblastic meningioma. However, it should be recalled that the tumor was comparatively small and was covered by intact arachnoid. A small angioblastic meningioma of the left frontal lobe without dural attachment has been reported by Wolf and Cowen,[12] case 4. The tumors attached to the dura are larger than these two neoplasms. The situation seems best explained by assuming that all were originally confined to the leptomeninges, as was the tumor in case 2. Enlargement of the neoplasm would lead to a break in the continuity of the layer of arachnoid cells. As discussed elsewhere,[10, 13] the dura participates in the repair of the defect thus produced. Because of this, any tumor which leads to discontinuity of the superficial layer of the arachnoid

becomes attached to the dura, no matter what its cell type is. Dural attachment of angioblastic meningiomas seems to depend on the production of a defect in the outer layer of the arachnoid with participation of tissue from the dura in filling the defect.

There are no essential differences in the histology of angioblastic meningiomas and hemangioblastomas of the brain. Each is composed in part of blood vessels of considerable size; in part, of more cellular tissue in which the only blood vessels are a few small capillaries. By the same sequences as those described in peripheral sclerosing hemangiomas, increased stroma, hemosiderin and fat make their appearance both in hemangioblastomas of the brain substance and in angioblastic meningiomas. Neuroglia occurs in both tumors; its source in hemangioblastomas has already been discussed.

Since neuroglia is not found in the brain envelopes, it has been suggested that the neuroglia of angioblastic meningiomas makes its appearance as the result of a congenital glial heterotopia in the meninges and that it forms, with the blood vessels, a complex congenital overgrowth of tissues. Although the angioblastic meningiomas for the most part push the underlying brain ahead of them, they extend into it by direct invasion for a short distance, with the result that portions of brain tissue come to be included within the tumor. The neurons in such areas are destroyed but the astrocytes survive and continue to grow, responding to the tumor so as to form a portion of its stroma. When further extension of the neoplasm takes place, the astrocytes persist and finally vessels of the tumor, neuroglia and collagen fibers are mixed together (Fig. 10). These histological sequences are identical with those encountered at the margins of hemangioblastomas of the brain substance.

It was definitely stated by Percival Bailey, Cushing and Eisenhardt [4] that the term *angioblastic meningioma* connotes a meningeal tumor of vascular origin and not a highly vascular neoplasm of arachnoid cells. In spite of this, various writers have included in their series tumors of both types or have regarded all the angioblastic meningiomas as vascular, cellular tumors originating in arachnoid cells (*vide* Bergstrand and Olivecrona [14]). The presence of a single psammoma body or of a few arachnoid cells in an angioblastic meningioma [12] represents the inclusion of tissue of the adjacent arachnoid by the expanding tumor. The neoplasms arising in arachnoid cells and commonly called *meningiomas,* no matter how vascular, are fundamentally different from the angioblastic meningiomas.

It is not necessary to postulate unique features of the cells from which angioblastic meningiomas arise to account for any of the histological features of the neoplasms. The histological features considered

to set this group apart from other tumors of blood-vessel origin can be explained by the progressive tissue changes of sclerosis. The study of blood-vessel tumors of the central nervous system would be greatly simplified if it were recognized that the tumors now called angioblastic meningiomas and those called hemangioblastomas of the brain substance are sclerosing hemangiomas of the central nervous system.

The histological sequences which take place in sclerosing hemangiomas of the brain and its coverings are reflected in the gross appearances of the tumors. In the portions where lipoid-filled phagocytes are abundant, the tissue has a yellow color. There is a brownish tint where hemosiderin has accumulated in large quantities. For this reason the otherwise reddish gray tumors become mottled with yellow, brown and orange areas. This gross appearance in a tumor, in and about which there are many very large blood vessels and blood-containing spaces, permits the identification of sclerosing hemangiomas of the central nervous system when they are exposed at operation. In case 1, the surgeon described the lesion at the first operation as red in color. Yellow areas were seen when the tumor was again exposed 13 years later. While it is possible that the yellow areas were overlooked at the first operation, the observation offers some confirmatory evidence that these fat deposits gradually make their appearance as the result of progressive tissue changes in hemangiomas.

The recognition of these tumors during a surgical procedure has a bearing on their management. In view of the speculation whether peripheral sclerosing hemangiomas may be an indication of a systemic disturbance in lipoid metabolism, it seems worth while to state again that the accumulation of fat in these tumors, central or peripheral, is not the result of a generalized disorder but of local tissue reactions. The sclerosing hemangiomas both of the meninges and of the brain substance extend into the adjacent brain instead of pushing the nervous tissue away, as do the meningiomas of arachnoid cell origin. For this reason, some of the grossly normal-appearing brain tissue about the sclerosing hemangiomas should be removed along with the tumors if complete extirpation is to be accomplished.

The progressive sequences of tissue behavior involved in sclerosing hemangiomas wherever found are in general to be regarded as regressive in character. On the other hand, the vessels of the neoplasms may continue to grow and especially the endothelial cells between the vessels may proliferate without the formation of vascular channels. Mitoses are usually found in these tumors and are abundant in some of the specimens. For this reason, sclerosis in hemangiomas is not in itself assurance of complete quiescence of the tumor cells. Enlargement

of the lesions may be due either to continued growth of neoplastic cells or to the process of sclerosis itself, on account of the increase in the amount of stroma and the accumulation of fat and hemosiderin.

The progressive tissue changes in sclerosing hemangiomas of the central nervous system indicate the extent to which the morphology of tumors may be modified by secondary histological sequences. When these are taken into consideration, many of the difficulties in morphological interpretation are removed and a more accurate definition of histogenetically related groups of tumors is made possible.

Summary

The neoplasms of blood-vessel origin in the central nervous system have been divided into two groups: the hemangioblastomas, arising in the brain substance and by far most frequently in the cerebellum; and the so-called angioblastic meningiomas. The presence of phagocytes containing fat and hemosiderin as well as of neuroglia in tumors of both groups have tended to set them apart from hemangiomas outside the central nervous system.

Study of a series of hemangioblastomas of the brain substance indicates that they may undergo a process of sclerosis entirely comparable to that found in hemangiomas of the skin and other organs. The sclerosis comes about by an increase in stroma, in large part collagenous connective tissue but in part neuroglia. In other words, both mesodermal and ectodermal supporting tissue participates in the process. The occlusion of blood vessels resulting from the increase in stroma is accompanied by an accumulation of lipoid material and hemosiderin in phagocytic cells of endothelial origin. Since the sclerosis may be absent, slight or marked, many apparently different tumors may be shown to have a common origin.

The so-called angioblastic meningiomas are hemangiomas of the meninges in which a similar process of sclerosis has occurred. These tumors infiltrate the brain to some extent; in this way, neuroglia comes to participate in the progressive tissue changes occurring in the hemangiomas of meningeal origin.

There is no fundamental difference between the two groups of tumors usually called hemangioblastomas of the brain and angioblastic meningiomas. They are both sclerosing hemangiomas of the central nervous system.

The recognition of these sequences in hemangiomas of the brain and its coverings has a bearing on the operative management of the lesions.

The study of the progressive tissue changes in sclerosing hemangiomas of the central nervous system has been facilitated by observation

of similar changes in hemangiomas of the skin and other organs. This is one of the many instances in which a lesion apparently unique to the brain can be shown to have its exact counterpart in lesions of other viscera when account is taken of the differences in the normal structural patterns of the organs concerned.

REFERENCES

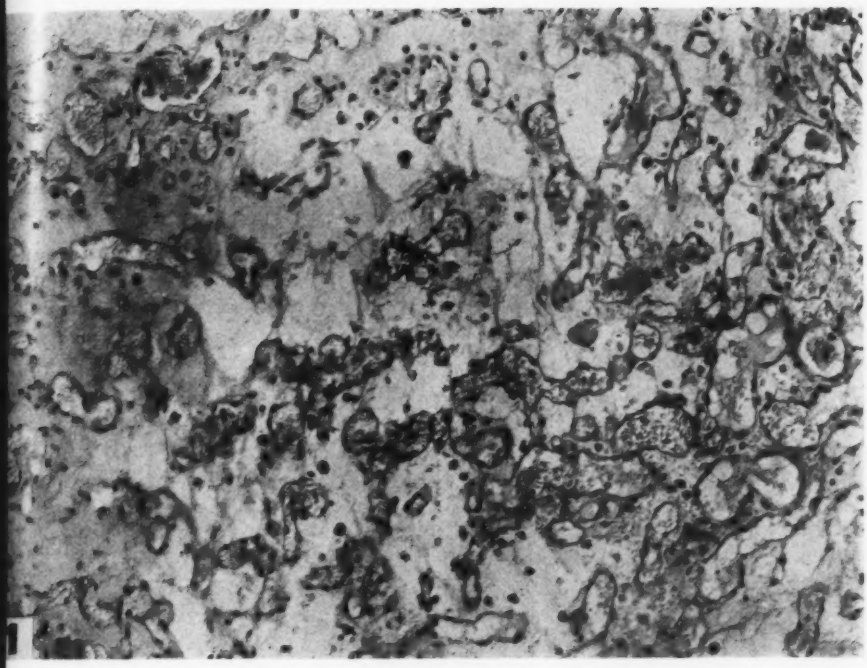
1. Wolbach, S. B. Hemangio-endotheliomata giving the histological appearances of "giant cell xanthoma." *Ztschr. f. Krebsforsch.*, 1913, **12**, 440–441.
2. Gross, R. E. Sclerosing hemangioma. Its relationship to xanthomas and certain pigmented lesions of the skin. (To be published.)
3. Cushing, Harvey, and Bailey, Percival. Tumors Arising from the Blood-Vessels of the Brain. Charles C. Thomas, Springfield, Ill., & Baltimore, Md., 1928.
4. Bailey, Percival; Cushing, Harvey, and Eisenhardt, Louise. Angioblastic meningiomas. *Arch. Path.*, 1928, **6**, 953–990.
5. Sargent, Percy, and Greenfield, J. G. Haemangeiomatous cysts of the cerebellum. *Brit. J. Surg.*, 1929–30, **17**, 84–101.
6. Mallory, F. B., and Parker, Frederic, Jr. Reticulum. *Am. J. Path.*, 1927, **3**, 515–525.
7. Wolbach, S. B. Controlled formation of collagen and reticulum. A study of the source of intercellular substance in recovery from experimental scorbutus. *Am. J. Path.*, 1933, suppl. **9**, 689–699.
8. Courville, C. B., and Abbott, K. H. The angioblastic group of meningiomas. A study of thirteen verified cases. *Bull. Los Angeles Neurol. Soc.*, 1940, **5**, 47–72.
9. Globus, J. H. Meningiomas. Origin, divergence in structure and relationship to contiguous tissues in light of phylogenesis and ontogenesis of the meninges, with suggestion of a simplified classification of meningeal neoplasms. *Arch. Neurol. & Psychiat.*, 1937, **38**, 667–712.
10. Bailey, O. T. Histologic sequences in the meningioma, with a consideration of the nature of hyperostosis cranii. *Arch. Path.*, 1940, **30**, 42–69.
11. Barnard, W. G., and Walshe, F. M. R. Capillary haemangeioma of cerebrum. *J. Path. & Bact.*, 1931, **34**, 385–387.
12. Wolf, Abner, and Cowen, David, Jr. Angioblastic meningiomas. Supratentorial hemangioblastomas. *Bull. Neurol. Inst. New York*, 1936, **5**, 485–514.
13. Bailey, O. T. Relation of glioma of the leptomeninges to neuroglia nests. Report of a case of astrocytoma of the leptomeninges. *Arch. Path.*, 1936, **21**, 584–600.
14. Bergstrand, H., and Olivecrona, H. Angioblastic meningiomas. *Am. J. Cancer*, 1935, **24**, 522–530.

DESCRIPTION OF PLATES

PLATE 1

FIG. 1. A hemangioblastoma of the central nervous system, without evidence of sclerosis. The tumor is composed of thin-walled capillaries separated from one another by a very delicate and edematous stroma. There are no cells containing hemosiderin or fat. Hematoxylin and eosin stain. × 150.

FIG. 2. A hemangioblastoma of the central nervous system with evidence of sclerosis. The capillaries are separated from one another by a cellular tissue. Mallory's phosphotungstic acid hematoxylin stain. × 150. (Fig. 4 shows the character of the cells of this tumor at higher magnification.)



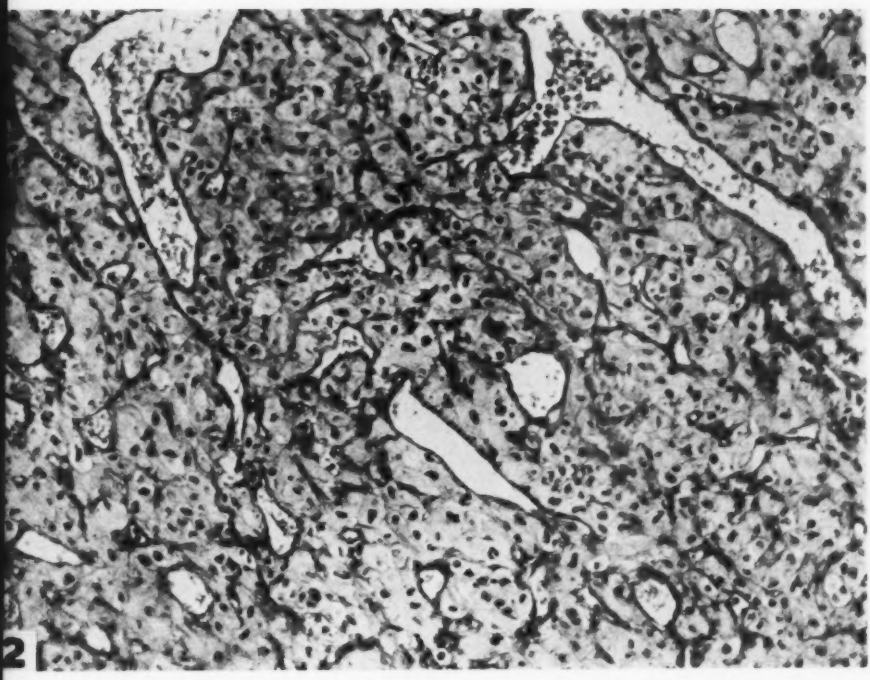
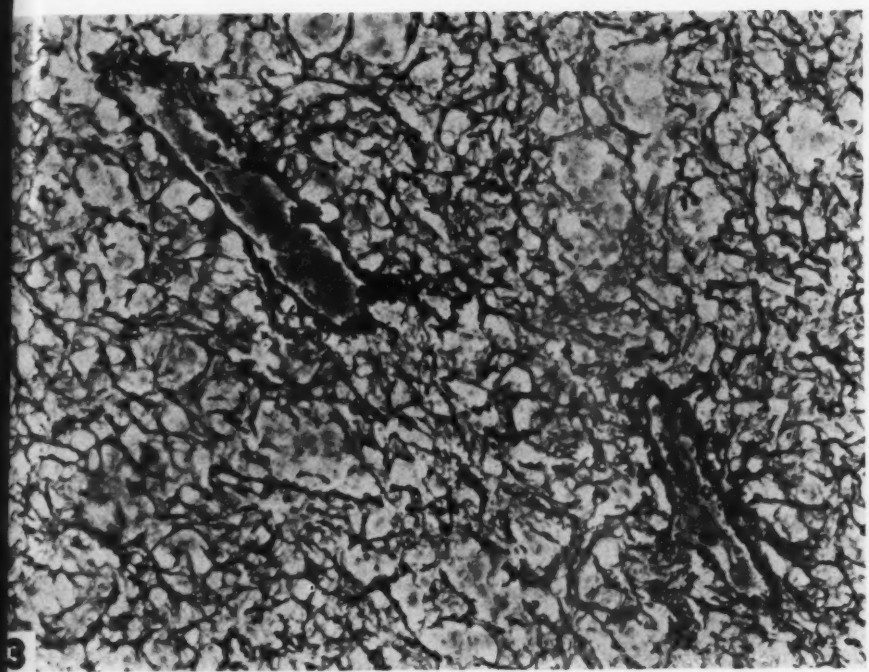


PLATE 2

FIG. 3. The reticulum in a sclerosing hemangioblastoma of the cerebellum. Two capillaries have been clearly outlined by the stain. A delicate reticulum extends among the cells of the intervascular tissue. Laidlaw's method for reticulum. × 300.

FIG. 4. The character of the cells in the tumor shown in Figure 2. Around the thin-walled capillaries there are numerous cells with nuclei resembling those of endothelial cells. Their cytoplasm contains fine vacuoles, due to the presence of fat. Mallory's phosphotungstic acid hematoxylin stain. × 900.



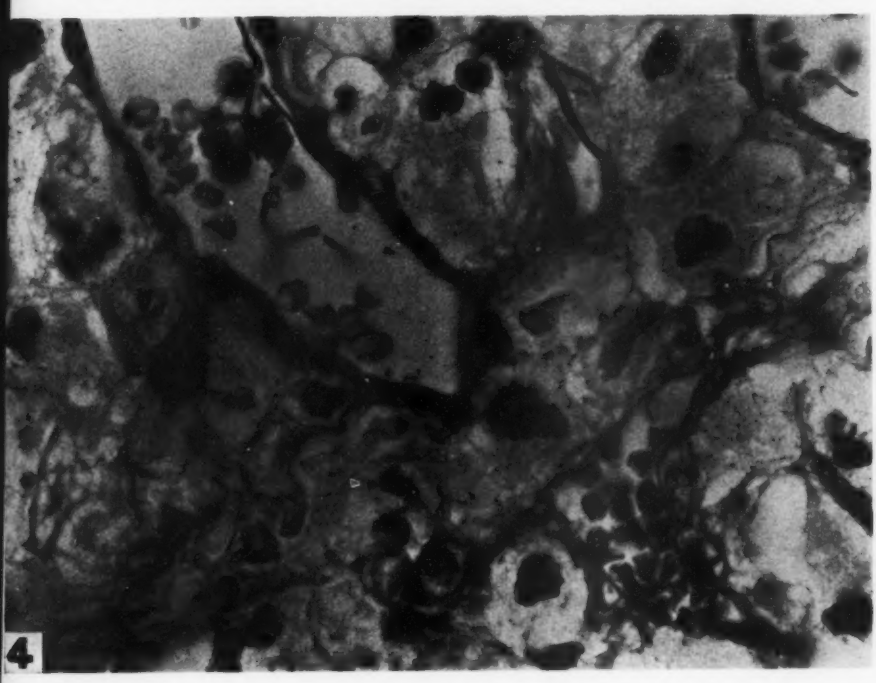


PLATE 3

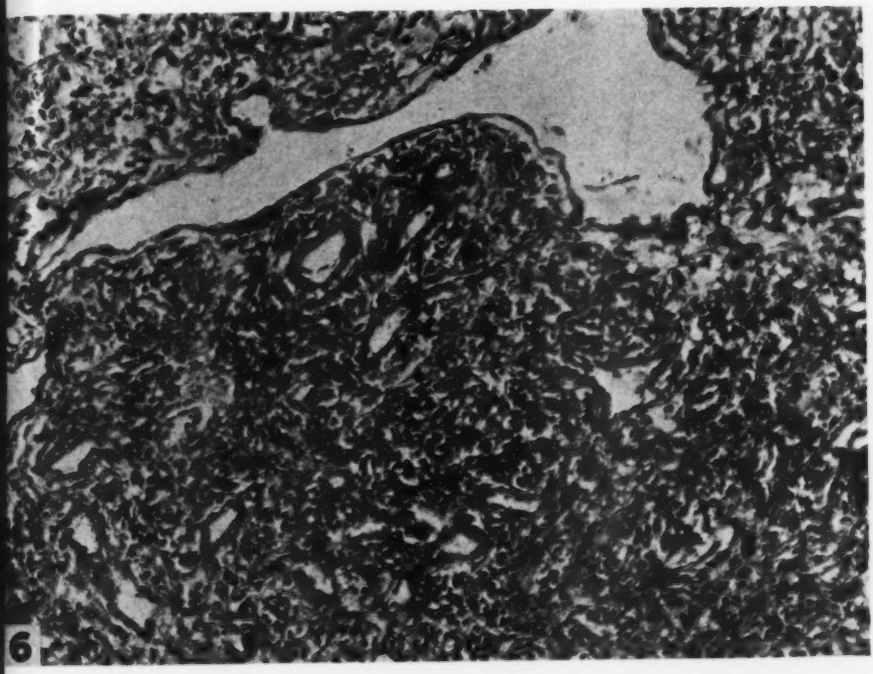
FIG. 5. The relations of the tumor in case 2 to surrounding structures. In the attachment to the meninges, the lack of cyst formation and the compression of the underlying brain, it resembles an angioblastic meningioma. The location in the cerebellum and the absence of attachment to the dura are characteristics usually associated with hemangioblastomas of the brain substance. Mallory's phosphotungstic acid hematoxylin stain. × 5.5.

Bailey and Ford Hemangiomas of the Nervous System

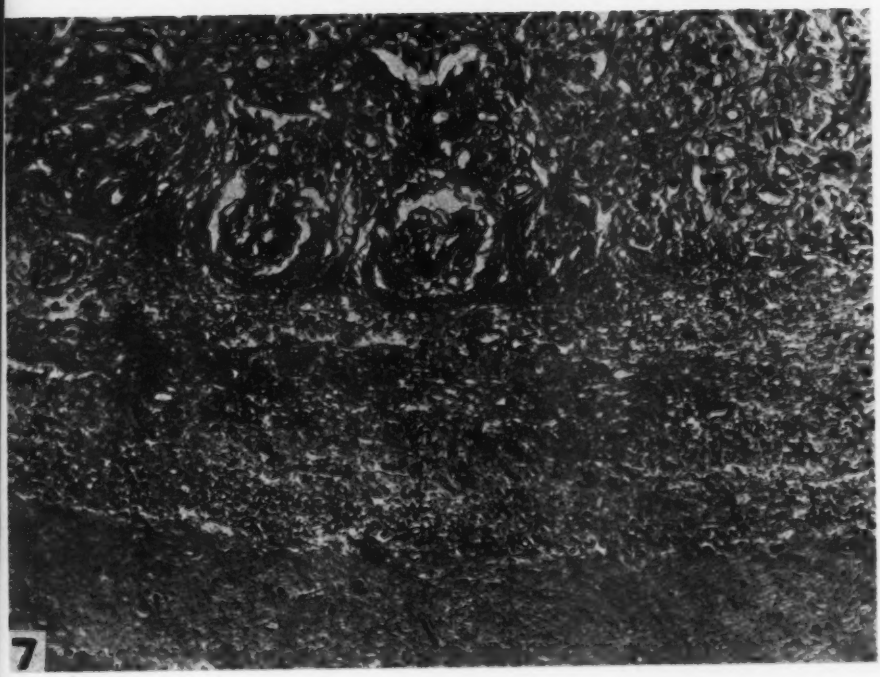
PLATE 4

FIG. 6. The tumor in case 2 is composed of blood vessels of various sizes separated from one another by cellular tissue. The resemblance to the tissue shown in Figure 2 is striking. Mallory's phosphotungstic acid hematoxylin stain. × 150.

FIG. 7. Case 2. This shows the edge of the tumor in one region in which it infiltrates the substance of the cerebellum. Mallory's phosphotungstic acid hematoxylin stain. × 150.



6



7

Plate 5

In Figures 8 to 11, the cells in the intervascular tissue are shown as they appear in sections stained by various technics.

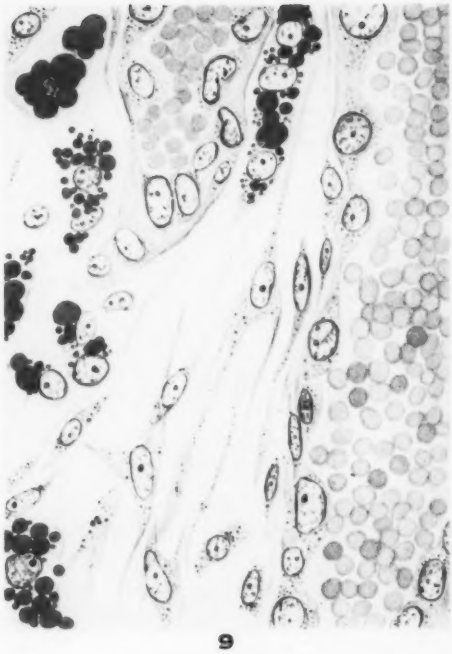
Fig. 8. Case 2. Some of the cells in the intervascular tissue contain hemosiderin. Mallory's ammonium sulfide-potassium ferricyanide method for iron. × 697.

Fig. 9. Case 2. Other cells in the intervascular tissue contain fat. Scharlach R stain. × 697.

Fig. 10. Case 2. In the region in which the neoplasm has infiltrated the cerebellum, astrocytes are incorporated in the tumor. This is representative of many fields in which capillaries, collagenous tissue and astrocytes are mixed together in a haphazard manner. Mallory's phosphotungstic acid hematoxylin stain. × 697.

Fig. 11. Case 2. The reticulum outlines a small capillary in the intervascular tissue. A delicate network of these fibers extends among the phagocytic cells in the region. Foot's method for reticulum. × 697.

8



9

10

11

Bailey and Ford

Hemangiomas of the Nervous System

THE PRODUCTION OF CARDIAC AND RENAL LESIONS IN RATS BY A DIET EXTREMELY DEFICIENT IN POTASSIUM *

RICHARD H. FOLLIS, JR., M.D., ELSA ORENT-KEILES, SC.D., and E. V. MCCOLLUM, PH.D.
(From the Department of Pathology, The Johns Hopkins Medical School, and the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.)

In 1937, Schrader, Prickett and Salmon [1] described pathological alterations in the heart, kidneys, pancreas and intestines of rats which had been placed on a diet deficient in potassium. More recently, by restricting the dietary potassium, Thomas, Mylon and Winternitz [2] have confirmed the cardiac lesions reported by Schrader, Prickett and Salmon in rats, though they failed to note any change in the other tissues. They were also able to produce the same myocardial lesions in hogs. However, they concluded that both in the latter animals as well as in rats the development of the lesions was dependent on a diet not only deficient in potassium but in vitamin B_6 as well. Sykes and Alfredson [3] have noted electrocardiographic changes in calves on diets of low potassium content.

The present study deals with the pathological alterations in the tissues of rats placed on a diet adequate in all known respects save in its content of potassium (0.01 per cent). The composition of this diet was as follows: wheat gluten, 4.0; lactalbumin (highly purified), 10.0; gelatin, 4.0; salts, no. 21, 4.6; butter fat (purified), 8.0; dextrose to 100 per cent; viosterol, 15 drops per Kg. of diet; liver concentrate (Lederle), approximately 3.5 gm. of liver per rat per day; thiamin, 20 γ per rat per day, and vitamin E concentrate, 3 mg. per rat per day. The growth, behavior and metabolism of animals placed upon this diet are being reported elsewhere by Orent-Keiles and McCollum.[4]

MATERIALS AND METHODS

Ninety rats of the McCollum strain, about equally divided as to sex, have been examined. Their ages, when placed upon the potassium-low diet, varied from 25 to 28 days. They were killed after being on the diet from 4 to 327 days. Approximately one-half of the animals served as controls and received the potassium-supplemented basal diet either *ad libitum* or in amounts restricted to the intake of the potassium-deficient animals. The latter precaution was taken to exclude, as far as possible, any rôle inanition might have in producing the changes

* This work has been supported by a grant to the Department of Biochemistry, School of Hygiene and Public Health, from the Rockefeller Foundation.
Received for publication, April 24, 1941.

observed. Seven of the animals were born from mothers that had been placed on the potassium-low diet 10 days before their birth and had continued on the diet during suckling. They remained on the diet after weaning as long as 67 days of age.

The animals were killed with ether and autopsied immediately. Tissues were placed in Zenker-formaldehyde solution and in a 4 per cent solution of formaldehyde alone. After imbedding in paraffin, they were stained with hematoxylin and eosin. Bones were decalcified in 5 per cent nitric acid. Since it seemed inadvisable to examine all tissues from every animal, a preliminary group of animals was studied thoroughly and then those organs which showed any deviation from the normal controls were studied more minutely in other animals in order to trace the development and course of the changes. The following tissues were studied: heart, lung, liver, spleen, pancreas, adrenal, kidney, esophagus, stomach, intestine, ovary, uterus, vagina, testis, seminal vesicle, prostate, thyroid, parathyroid, thymus, arteries, skin, skeletal muscle, brain, hypophysis, bone and bone marrow. Only the positive gross and microscopical findings will be described. There were marked differences in the gonads and accessory sexual apparatus, thymus and bones of the animals fed *ad libitum* and of the potassium-deficient animals, but when the factor of inanition was ruled out by paired feeding these differences were not present.

Gross and Microscopical Changes

Heart

Grossly, changes were not seen in the heart before the third week. At 15 days, however, with the aid of binocular loupes, tiny, opaque grayish areas could be seen beneath the epicardium of some of the deficient animals. By the sixth week these lesions could be detected with the naked eye and from then on these areas were seen in practically all potassium-deficient animals. The controls showed nothing. The only other gross finding of note was a difference in the weight of the hearts of the deficient animals and their controls fed *ad libitum*. The ratio of heart weight to body weight indicated that on the whole the hearts of the former group were the larger, but interpretation of this is not accurate because we do not know how much of a rôle inanition was playing. However, some light was cast on the assumption that there was a true hypertrophy by the fact that in a group of seven animals (four deficient and three controls) on the paired feeding regimen for 87 days, the deficient animals weighed 38 per cent less than their controls, but their hearts weighed 13 per cent more than those of the latter.

Microscopically, the earliest change to be noted was a loss of striation of individual muscle fibers. This was seen either in isolated fibers or in groups of fibers after the animals had been on the potassium-low diet for 8 days. The hyaline fibers next lost their affinity for eosin and took on a grayish yellow color. After this they disappeared altogether, leaving an empty membrane. No fatty change could be demonstrated with the Scharlach R stain. Coincident with the change in the myofibrils was one which was found in the nuclei. As the fibers were undergoing necrosis the nuclei began to shrink and very soon underwent karyolysis or karyorrhexis. Associated with these changes there was an emigration of leukocytes into such areas (Fig. 1). In the early stages there were polymorphonuclear leukocytes while later mononuclear phagocytes predominated. There was a tendency for the earlier lesions to be more numerous beneath the epicardium and endocardium but necrotic fibers were found also in the center of the ventricular walls. No reaction was ever seen on the surface of the epicardium or endocardium. Lesions were scanty in the auricular musculature. Mural thrombi were not found in the ventricular chambers. Aside from the leukocytic infiltration in the zones of necrosis, no other cellular response was found. There were no perivascular accumulations of leukocytes, nor any changes in the vessels themselves.

From the eighth to the fifteenth day these lesions increased both in size and in number, so that at 2 weeks numerous large areas, covering as much as two low-power microscopical fields in greatest diameter, could be found (Fig. 2). In some animals the process took on the appearance of a diffuse myocarditis with widely scattered necrotic fibers surrounded by leukocytes. Other areas suggested the lesions that are encountered in human diphtheria. In the ensuing weeks the involvement became more extensive so that by the sixth week the necrotic foci were extremely numerous. By this time, too, there was an increasing proliferation of connective tissue at the sites where earlier lesions had been present. These scarred regions consisted of single fibers or groups of intact muscle fibers separated by connective tissue cells and bundles of collagen (Fig. 3). Scattered about, too, were mononuclear leukocytes. It was interesting to note old scarred lesions close to muscle fibers which were undergoing the necrotic changes as described. From the sixth week to the end of the period of observation in the 47th week the scarring became more extensive, while in contrast there were fewer fresh lesions. However, it must be noted that lesions indicating recent myocardial damage were found in animals on the deficient diet as long as they were observed (327 days).

When the animals which had been born to rats on the deficient diet

were examined, the same myocardial lesions were seen. At 4 weeks of age there were fresh necroses together with scars, and these same changes were found in animals examined in the sixth, eighth and tenth weeks after birth. Because of the report [5] of cardiovascular lesions in calves due to magnesium deficiency, a group of animals was given magnesium, in excess of that in the basal diet. This supplement had no effect on the development of the lesions.

In summary, in the myocardium of all animals which had been on the potassium-deficient diet 8 days or longer there occurred necrosis of the muscle fibers. Such areas then became infiltrated with leukocytes and healing took place with a proliferation of connective tissue. Similar lesions were not found in the control animals on the same diet plus an adequate potassium intake.

Kidneys

Grossly, the most prominent change in the kidneys was an increase in size of those of the potassium-deficient animals. Most marked from the sixth week on, the difference was most impressive in the paired feeding group where, although the deficient animals weighed less than their restricted controls, the kidneys of the former group weighed more than those of the latter animals. This was not only true of the wet weight but of the dry weight as well. The surfaces were finely pitted and they were paler than the corresponding control organs.

Microscopically, the first change to be noted appeared after the animals had been on the potassium-deficient diet for 8 days. In the cytoplasm of the cells of the convoluted tubules small vacuoles appeared between the basement membrane and the nucleus. When frozen sections were stained with Scharlach R, these droplets were found to be lipid in nature (Fig. 4). When examined through the polarizing microscope they were not doubly refractive. By the 14th day the lipid had increased so that most of the cells of the convoluted tubules contained it. However, by this time other changes were present. Necrotic epithelial cells as well as swollen cells with poorly staining nuclei could be found. These cells became detached from the basement membrane and lay as pink hyaline masses in the lumina of the tubules. Coagulated pink-staining material was found in other lumina. There were also dilated tubules lined by flattened epithelium, which had apparently regenerated (Fig. 5). A few multinucleated tubular epithelial cells were found. In one kidney at 15 days a few "calcified" epithelial cells were found. During this early period no changes could be detected in the glomeruli or blood vessels.

By the sixth week there were many dilated tubules lined by flattened

epithelium (Figs. 6 and 7). At this time an additional feature was added to the picture. In a few tubules there appeared a blue-staining, granular material. One or two such casts could be found in the tubules in each section and as time went on they became more numerous. A few calcified epithelial cells were noted also. The only other finding of importance was an increase in size of the individual epithelial cells when compared with the same cells from control animals on the paired feeding regime. At the twelfth week and from then on to the end of the experiment the dilatation became more marked. The amount of "calcified" material did not increase greatly or reach the degree reported in magnesium deficiency [6] and following parathormone administration.[7]

In summary, the kidneys showed necrosis of the tubular epithelium, dilatation of the tubules and later deposition of "calcified" material in the lumina of some of the tubules.

Discussion

By feeding rats a diet extremely low in potassium but adequate in all other known constituents, necrosis of the cardiac musculature and damage to the renal tubular epithelium have been produced. The other tissues appeared normal. These myocardial lesions are apparently exactly similar to those observed in rats by Schrader, Prickett and Salmon [1] and in both rats and hogs by Thomas, Mylon and Winternitz.[2] However, although the cardiac lesions are alike, there are discrepancies noted in the other organs in these three studies. Schrader, *et al.* also described alterations in the kidneys similar to those here described and, in addition, lesions in the pancreas and intestine. They failed to eliminate the factor of partial inanition. Thomas and his associates noted lesions in the heart only. The compositions of the diets used in these three studies differ. McCollum, Orent-Keiles and Day[8] have already criticized the diet employed by Schrader, Prickett and Salmon as being inadequate in other dietary essentials aside from potassium. The diets used by Thomas, Mylon and Winternitz were deficient in factors of the vitamin B complex other than thiamin, riboflavin and B_6. In addition, their diet I contained an excess of cod liver oil and was deficient in vitamin E. These workers concluded that potassium was not the only factor in producing the lesions but that a deficiency of vitamin B_6 was also necessary. These differences in the diets employed may be sufficient to explain the discrepancies in the lesions produced in these experiments.

Since the classic studies of Ringer,[9] in 1882, the importance of potassium in the normal physiology of the heart has been well recognized.

The exact mechanism for the production of cardiac lesions by diets low in potassium is quite obscure. Unfortunately, at this time, we do not know how much the blood-potassium level was affected in the rats on our potassium-low diet. The potassium content of the heart muscle, however, was 35 per cent lower than that of the controls. When one attempts to apply these experimental findings to disease in man associated with low blood potassium there is very little available information. In familial periodic paralysis there is a low blood potassium during attacks and at such time electrocardiographic changes have been described.[10] Another possibility which comes to mind is the lowering of serum potassium in patients with Addison's disease to whom an excessive amount of desoxycorticosterone acetate had been administered. Here, cardiac failure has been described,[11] though it may be due to some factor other than lowering of the serum potassium. To our knowledge no histological studies on such hearts have been reported.

It is worth while pointing out that the cardiac musculature was the only type affected. The striated myofibrils as well as the involuntary fibers were not damaged, even though the potassium content of the voluntary muscle was lower than that of the myocardium.

The changes in the kidneys are fairly characteristic of the so-called "necrotizing nephroses" which can be produced by various chemical poisons, such as salts of mercury or uranium or bacterial poisons as well. The exact mechanism for the renal changes in this experiment is obscure. It was hoped that metabolic studies might help in explaining the pathogenesis of the lesions. In the deficient animals the potassium excretion was very low during the first 3 weeks and after this period practically none of this cation was found in the urine. There was a diminished excretion of magnesium from the first to the seventh week in the potassium-deficient animals as well as a diminution in the urinary output of phosphate as compared with the normal controls. These data are difficult to interpret. Renal lesions have been reported in rats on a magnesium-deficient diet, though the various observers do not agree as to the exact renal changes. Greenberg, Lucia and Tufts [6] have described extensive calcium deposition in the renal tubules with atrophy of the tubular epithelium. We have recently been able to examine the kidneys of three rats that had been placed on a magnesium-deficient diet for 35 days. In these there was necrosis of the epithelium of the convoluted tubules. Calcified material was found in only one of the animals. The change was much more severe than we observed in the potassium-deficient animals, nor was tubular dilatation prominent. McKay and Oliver [12] have observed lesions in rats which bear a striking similarity to those we have observed by feeding an excess of inorganic

phosphate. In our animals, as previously noted, there was a diminution in the output of phosphate. The exact nature of the mechanism involved in the renal tubular necrosis in the potassium-deficient animals is obscure.

Summary

By the utilization of a diet adequate in all known respects except for an extremely low content of potassium (0.01 per cent) lesions have been produced in the heart and kidneys of rats restricted to this diet. In the heart, necrosis of the myocardial fibers followed by scarring has been described. Necrosis of the renal tubular epithelium with regeneration and tubular dilatation was also observed. The exact mechanism for the production of these lesions has not been elucidated.

Note: The technical work was performed by Miriam C. Reed and the photomicrographs were made by Milton Kougl.

References

1. Schrader, G. A.; Prickett, C. O., and Salmon, W. D. Symptomatology and pathology of potassium and magnesium deficiencies in the rat. *J. Nutrition*, 1937, **14**, 85–109.
2. Thomas, R. M.; Mylon, E., and Winternitz, M. C. Myocardial lesions resulting from dietary deficiency. *Yale J. Biol. & Med.*, 1940, **12**, 345–360.
3. Sykes, J. F., and Alfredson, B. V. Studies on the bovine electrocardiogram. I. Electrocardiographic changes in calves on low potassium rations. *Proc. Soc. Exper. Biol. & Med.*, 1940, **43**, 575–579.
4. Orent-Keiles, Elsa, and McCollum, E. V. Potassium in animal nutrition. *J. Biol. Chem.*, 1941, **140**, 337–352.
5. Moore, L. A.; Hallman, E. T., and Sholl, L. B. Cardiovascular and other lesions in calves fed diets low in magnesium. *Arch. Path.*, 1938, **26**, 820–838.
6. Greenberg, D. M.; Lucia, S. P., and Tufts, E. V. The effect of magnesium deprivation on renal function. *Am. J. Physiol.*, 1938, **121**, 424–430.
7. Chown, Bruce; Lee, Margaret; Teal, John, and Currie, Robert. On the experimental production of nephritis in rats by means of parathyroid hormone and of vitamin D. *J. Path. & Bact.*, 1939, **49**, 273–290.
8. McCollum, E. V.; Orent-Keiles, Elsa, and Day, H. G. The Newer Knowledge of Nutrition. The Macmillan Co., New York, 1939, ed. 5, pp. 201–202.
9. Ringer, Sydney. A further contribution regarding the influence of the different constituents of the blood in the contractions of the heart. *J. Physiol.*, 1882, **4**, 29–42.
10. Smith, W. A. Periodic paralysis. Report of two fatal cases. *J. Nerv. & Ment. Dis.*, 1939, **90**, 210–215.
11. Ferrebee, J. W.; Ragan, Charles; Atchley, D. W., and Loeb, R. F. Desoxycorticosterone esters. Certain effects in the treatment of Addison's disease. *J. A. M. A.*, 1939, **113**, 1725–1731.
12. McKay, E. M., and Oliver, J. Renal damage following the ingestion of a diet containing an excess of inorganic phosphate. *J. Exper. Med.*, 1935, **61**, 319–333.

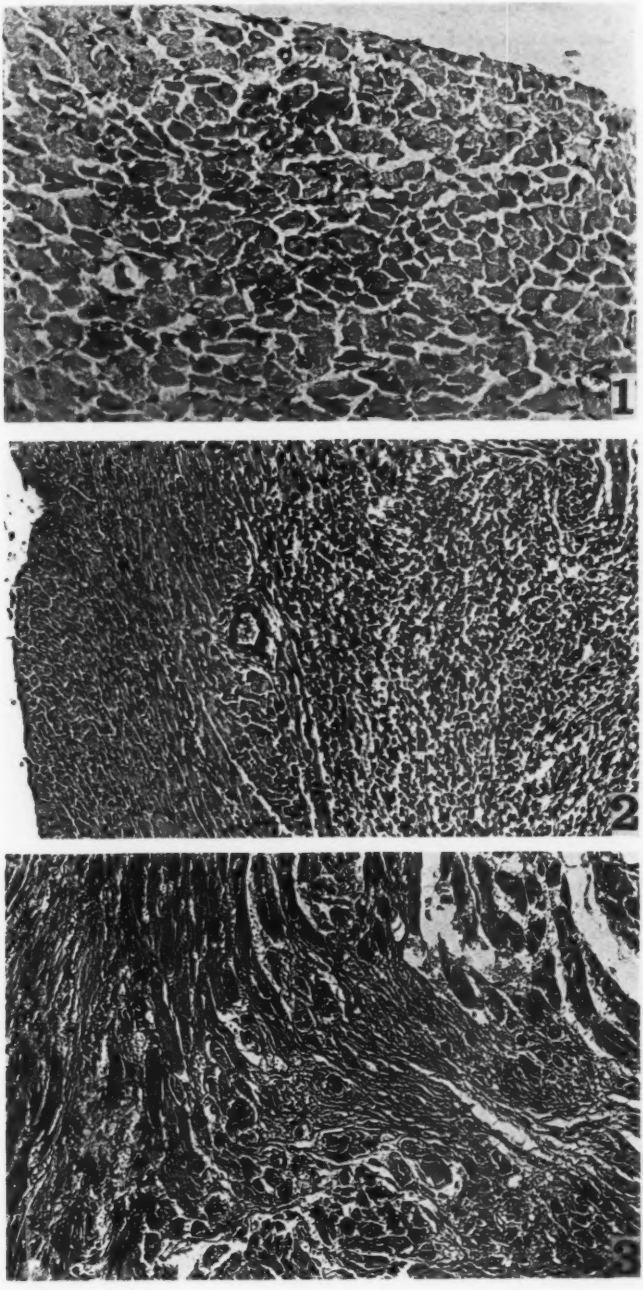
DESCRIPTION OF PLATES

PLATE 6

FIG. 1. Fresh lesion in the myocardium of a potassium-deficient animal showing leukocytic infiltration about necrotic muscle fibers. × 400.

FIG. 2. More extensive lesion in the myocardium of a potassium-deficient rat showing diffuse mononuclear infiltration after 15 days on the diet. × 100.

FIG. 3. Photomicrograph showing scarring in the myocardium of a potassium-deficient animal after being on the diet 87 days. × 100.



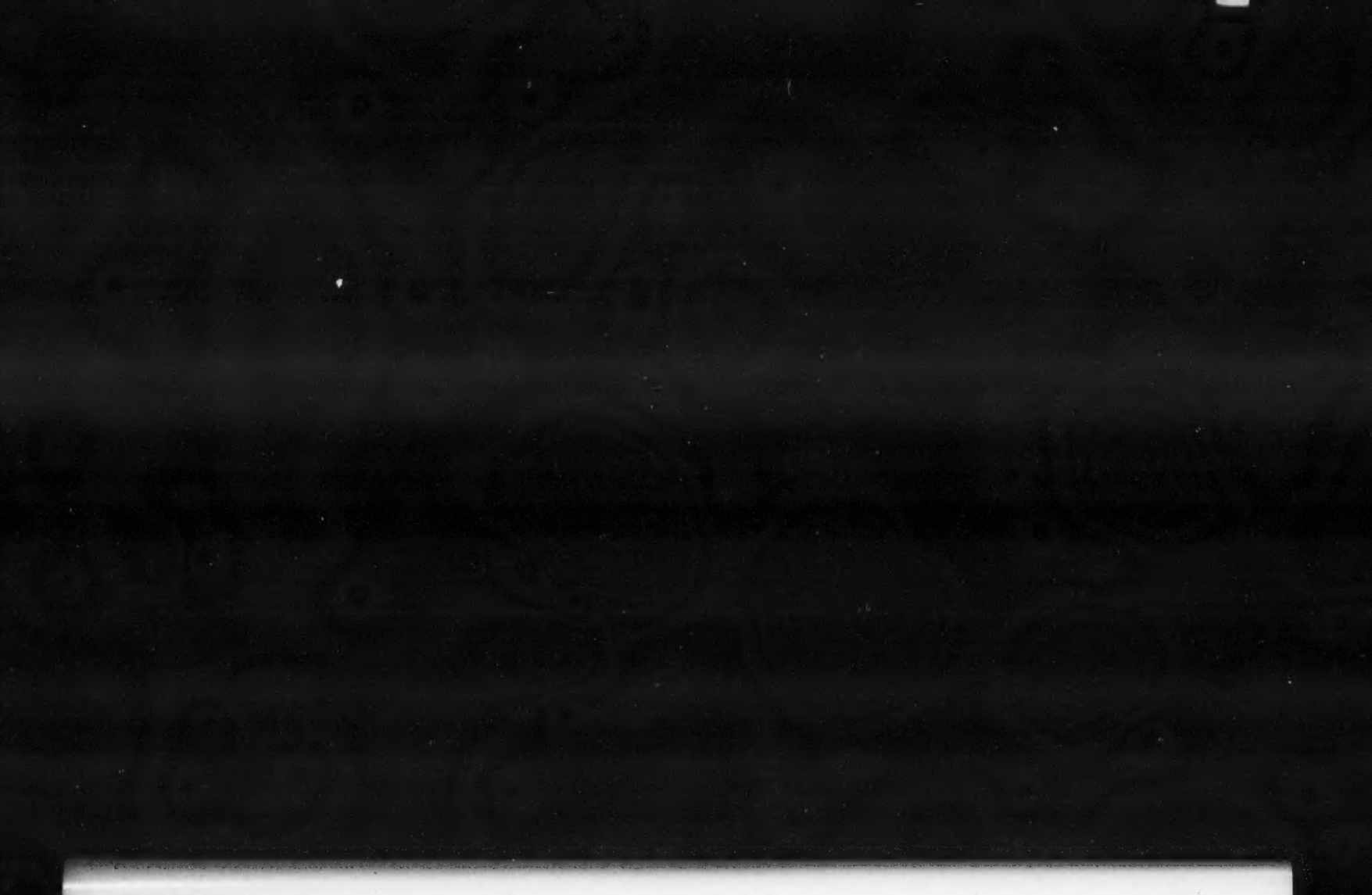
Follis, Orent-Keiles and McCollum Lesions with Potassium Deficiency

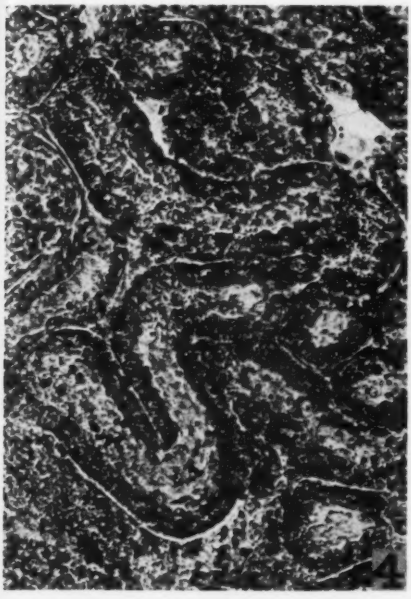
PLATE 7

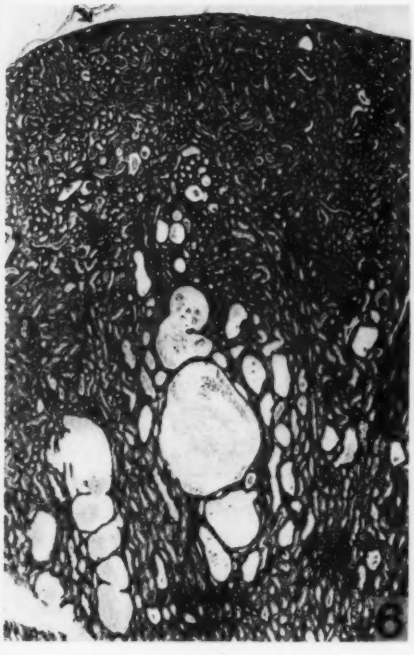
FIG. 4. Frozen section of kidney from a rat on the potassium-deficient diet for 13 days, showing lipid droplets in tubular epithelium (stained with Scharlach R). × 200.

FIG. 5. Photomicrograph showing vacuolated tubular epithelial cells and a dilated tubule containing necrotic cells and débris, from a rat on the potassium-deficient diet for 15 days. × 200.

FIGS. 6 and 7. Medium and low power views of kidney from a rat on the potassium-deficient diet for 6 weeks showing dilatation of the tubules. Arrows point to small collections of calcified material in the tubules. × 90 and × 15.







Follis, Orent-Keiles and McCollum Lesions with Potassium Deficiency

THE DEPOSITION OF CALCIUM IN THE HEARTS AND KIDNEYS OF RATS IN RELATION TO AGE, SOURCE OF CALCIUM, EXERCISE AND DIET *

LE ROY L. BARNES, Ph.D.
(From the Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.)

In this laboratory, stereoroentgenographs of some of the organs of our rats, taken at the time of autopsy, have proven useful in estimating the incidence and extent of calcium deposition. We have previously reported the use of this technic in connection with the study at autopsy of the rats from our retarded-growth experiments.[1,2] The high incidence of calcification which we found in some of the organs of those rats has led to the present study.

The data to be presented here were obtained from three experiments which were started during or near the end of the retarded-growth experiments.[1,2] Experiment I was one of several experiments conducted by McCay, Maynard, Sperling and Osgood [3] to study the effects of different levels of protein in the diet, and of exercise, on the life span of the rat. The complete diet of this group of rats is given in Table 3 of their paper. Here I shall mention only those variables in the experiment which seem to influence calcium deposition in the heart and kidneys. Experiment II was conducted by Bogart,[4] Asdell [5] and their co-workers to determine some of the effects of reproduction on the life span of the female rat. The diet used in this case was our stock diet.[6] Experiment III was conducted by McCay and associates and is still unpublished. The complete diet for this experiment is given in Table I.

CALCIFICATION OF THE HEART

Stereoroentgenographs of the hearts of the rats were made and when calcium was found, its approximate location was noted. Among the older rats a calcified region in the auricles was fairly common; in some cases calcification in the region of the valves could be seen, and in some, one or more regions of calcification were found in the ventricles. The three hearts shown in Figures 1, 2 and 3 are examples of calcified regions found in auricle, ventricle and valvular region respectively.

In all three experiments the incidence of calcification was found to increase with the age at death (Table II).

In experiment I,[3] in which protein, exercise and fatness were variables, stereoroentgenographs of 232 hearts were made at the time of

* Aided by a grant from the Rockefeller Foundation for research in longevity.
Received for publication, February 20, 1941.

autopsy. The average age at death for these animals was 636 days. About 42 per cent of the hearts showed at least one calcified region. Twenty-four per cent showed calcification in the auricles, 16 per cent in the ventricles and 12 per cent in the region of the valves.

The incidence of calcification of the heart in relation to the different dietary variables in this experiment showed no marked differences. However, the exercise variable combined with the level of food intake gave a result which may be significant. The hearts of 58 rats on unrestricted food intake and enforced exercise [3] showed an incidence of calcification of 55 per cent, while those of 58 rats not allowed to become fat but forced to exercise showed an incidence of only 36 per cent.

In experiment II [4] (the longevity-reproduction experiment) the average age at death for 287 females, from which hearts were obtained, was 663 days. Forty-three per cent of these hearts showed some calcification. Twenty-five per cent showed calcification in the auricles, 11 per cent in the ventricles and 11 per cent in the region of the valves.

TABLE I

Diets in Experiment III

	E	F	G	H	I	J
	(Per cent)	(Per cent)	(Per cent)	(Per cent)	(Per cent)	(Per cent)
Dry liver	12	12	12	12	12	12
Sucrose	10	10	10	10	10	10
Cellulose	2	2	2	2	2	2
Wesson oil	7	7	7	7	7	7
Yeast	5	5	5	5	5	5
Cod liver oil*	0.25	0.25	0.25	0.25	0.25	0.25
$CaCO_3$	0.33	0.33	0.33	—	—	—
$CaHPO_4 \cdot 2H_2O$	—	—	—	1.10	1.10	1.10
NaCl	0.70	0.20	1.50	0.70	0.20	1.50
Starch (cooked)	63.43	63.22	61.92	62.95	62.45	61.15

* Fortified cod liver oil (vitamin D, 800 I. U. per gm.; vitamin A, 6000 I. U. per gm.)

TABLE II

Incidence of Calcification of the Heart in Relation to Age at Death

Age range (days)	Experiment I male rats		Experiment II female rats		Experiment III male rats	
	Number of hearts	Incidence of calcification (per cent)	Number of hearts	Incidence of calcification (per cent)	Number of hearts	Incidence of calcification (per cent)
300–399	10	20	12	33	—	—
400–499	26	23	21	29	18	22
500–599	56	29	41	41	52	31
600–699	66	44	57	46	56	25
687–704*	—	—	97	38	69	49
700–799	51	50	26	35	17	47
800–899	16	75	21	62	4	50
900	7	86	12	75	—	—

* Rats killed accidentally by high temperature.

In Table III the groups of rats used in this experiment are arranged in the order of increasing incidence of calcification of the heart. The highest incidence appears in the group in which breeding was not started until the rats were nearly 300 days old, and the lowest incidence is found in the group which was castrated a short time after weaning. For details of the handling of the rats in this experiment, the paper by Bogart and co-workers [4] should be consulted.

In experiment III, where the variables were mineral supplement and exercise, stereoroentgenographs of the hearts of 216 male rats were made. The average age at death for these animals was 636 days. Here the incidence of cardiac calcification was 36 per cent. The auricles showed an incidence of calcification of 18 per cent; the ventricles, 13 per cent, and the valvular region, 9 per cent.

The results of this experiment suggest that the form of the mineral supplement affects the incidence of calcification. The hearts of 108 rats receiving the $CaCO_3$ supplement showed an incidence of calcification of 30 per cent and those of 108 rats receiving the $CaHPO_4 \cdot 2H_2O$ supplement an incidence of 43 per cent.

Half of the animals receiving the $CaCO_3$ supplement and half of those receiving the $CaPHO_4 \cdot 2H_2O$ supplement were given moderate enforced exercise.[3] The exercise seemed to have had no effect on the incidence of calcification of the heart among the 108 rats receiving the $CaCO_3$ supplement. In this group the incidence was 30 per cent for the 54 animals forced to exercise and 30 per cent for the 54 animals not forced to exercise. However, in the case of the 108 rats receiving the $CaPHO_4 \cdot 2H_2O$ supplement, the results suggest an exercise effect. The hearts of 54 rats from this group that received no enforced exercise showed an incidence of calcification of 57 per cent and those of the 54 rats with enforced exercise an incidence of only 27 per cent.

These two diets were analyzed for calcium and phosphorus. The diet containing the $CaCO_3$ supplement contained 0.25 per cent of cal-

TABLE III

Incidence of Calcification of the Heart in Longevity-Reproduction Experiment (Experiment II)

Group	Number of hearts	Average age at death	Incidence of calcification
		(days)	*(per cent)*
Castrates	50	666	33
Virgins	48	652	35
Bred normally	47	643	40
Gestation without lactation	47	652	47
Bred early	45	683	49
Bred late	48	681	52

cium and 0.27 per cent of phosphorus (by weight), giving a calcium-phosphorus ratio of 0.93. The other diet contained 0.38 per cent calcium and 0.59 per cent phosphorus, with a calcium-phosphorus ratio of 0.64.

Calcium Deposition in Kidneys

Figures 4, 5 and 6 show different types of renal calcification which have been found in rats in these experiments. Figure 4 shows rather extensive calcium deposition in the medulla of the kidney; Figure 5, a deposition in the pelvis and Figure 6, extensive calcification in the cortex.

In experiment I, stereoroentgenographs of 468 kidneys from 234 male rats showed 48 kidneys with calcified regions sufficiently large to be detected by this method. This gives an incidence of 10.3 per cent. The extent of this calcification varied from one small spot in the kidney to a few cases as extensive as those in Figures 4, 5 and 6. In this experiment 5 per cent of the kidneys showed calcification in the cortex of the kidney, 4 per cent in the medulla and 2 per cent in the pelvis-pyramid region.

If the different variables in the experiment are considered, the lowest incidence of renal calcification (4 per cent) is found in the group of 57 rats with restricted food intake and no enforced exercise, while the group showing the highest incidence (17 per cent) was a group of 58 rats unrestricted in food intake and forced to exercise.

Considering the dietary variables only, the lowest incidence of renal calcification (7 per cent) is found in a group of 57 rats on the high casein diet, and the highest incidence (16 per cent) in a group of 55 rats on the low-liver diet.

There is also a suggestion that the age at which the rat was shifted from the stock diet to the experimental diet had an effect on the incidence of renal calcification. The kidneys of 238 rats started on this experiment between the ages of 204 and 280 days showed an incidence of calcium deposition of 6 per cent, while the kidneys of 230 rats started on the experiment between the ages of 420 and 431 days showed an incidence of 15 per cent.

In experiment II (longevity-reproduction), in which all of the rats had the stock diet,[6] the incidence of calcium deposition in the kidneys was low and the calcification was less extensive than in the kidneys of the rats of either of the other experiments. In this case stereograms were made of the kidneys of 283 female rats. Of the 566 kidneys examined, only 50 (9 per cent) showed any detectable calcium deposition. Two per cent of the kidneys showed calcium in the pelvis-pyramid

region, 4 per cent in the medulla and 3 per cent in the cortex. A comparison of the groups subjected to the different reproduction variables showed no marked differences.

In experiment III an examination of the stereograms of 432 kidneys from male rats showed an incidence of calcification of 18 per cent. Four per cent showed small calcium deposits in the pelvis-pyramid region, 13 per cent showed calcification in the medulla and 4 per cent showed some calcification in the cortex.

The exercise variable in this experiment seemed to have had no effect on calcium deposition in the kidneys. However, the mineral supplement variable had affected the calcium deposition in the kidneys. Of 216 kidneys from rats receiving the $CaCO_3$ supplement, only 9 per cent showed any calcium. Of the 216 kidneys from rats receiving the $CaHPO_4 \cdot 2H_2O$ supplement, 28 per cent showed calcium deposits.

Summary

1. This study shows, in all three experiments, that the incidence of calcification of the heart is an increasing function of the age at death.

2. The auricles are shown to be more common sites for calcium deposition than are the ventricles and the region of the valves.

3. Rats not allowed to become fat and forced to exercise show a lower incidence of calcification of the heart than those on unlimited food intake and enforced exercise.

4. The results suggest that the form of the mineral supplement has an effect on the incidence of calcification of the heart and that moderate enforced exercise may modify this effect.

5. In a large group of female rats all receiving the same diet but subject to reproduction variables, the highest incidence of calcification of the heart was found in a group in which breeding was delayed until middle life.

6. In the study of the kidney the medulla was found to be the most common site for calcium deposition.

7. Rats receiving a mineral supplement of $CaHPO_4 \cdot 2H_2O$ showed an incidence of renal calcification three times that shown by rats receiving a supplement of $CaCO_3$.

Note: The author is indebted to C. M. McCay and L. A. Maynard for helpful suggestions, to Gordon Ellis for calcium and phosphorus analyses of the diets, and to Gladys Sperling for help with the stereoroentgenographs.

References

1. Hummel, K. P., and Barnes, L. L. Calcification of the aorta, heart and kidneys of the albino rat. *Am. J. Path.*, 1938, 14, 121–124.

2. McCay, C. M.; Ellis, G. H.; Barnes, L. L.; Smith, C. A. H., and Sperling, Gladys. Chemical and pathological changes in aging and after retarded growth. *J. Nutrition,* 1939, **18,** 15–25.

3. McCay, C. M.; Maynard, L. A.; Sperling, Gladys, and Osgood, H. S. Nutritional requirements during the latter half of life. *J. Nutrition,* 1941, **21,** 45–60.

4. Bogart, R.; Sperling, G.; Barnes, L. L., and Asdell, S. A. The influence of reproductive condition upon growth in the female rat. *Am. J. Physiol.,* 1940, **128,** 355–371.

5. Asdell, S.; Bogart, R., and Sperling, Gladys. The influence of age and rate of breeding upon the ability of the female rat to reproduce and raise young. *Cornell University Agricultural Experiment Station,* 1941, memoir no. 238. (In press.)

6. Maynard, L. A. A diet for stock rats. *Science,* 1930, **71,** 192–193.

DESCRIPTION OF PLATE

PLATE 8

Roentgenographs of hearts and kidneys

FIG. 1. Heart, showing calcium deposition in auricle.

FIG. 2. Heart, showing calcium deposition in ventricle.

FIG. 3. Heart, showing calcium deposition in valvular region.

FIG. 4. Kidney, showing calcium deposition in medulla.

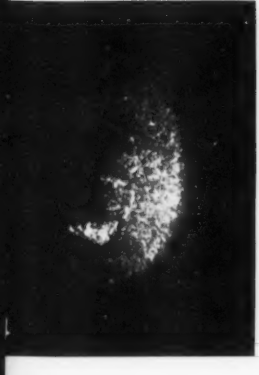
FIG. 5. Kidney, showing calcium deposition in pelvis.

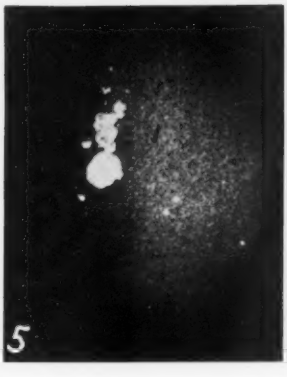
FIG. 6. Kidney, showing calcium deposition in cortex.

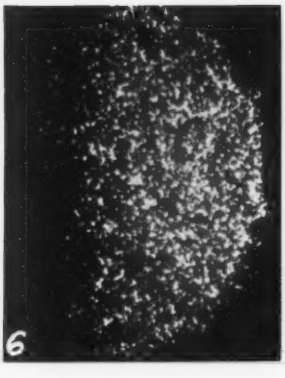
1

2

3




5


6

PATHOLOGICAL CHANGES IN NUTRITIONAL GASTRITIS IN RATS *

BENJAMIN N. BERG, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

In 1913, Fibiger [1] described lesions in the rumen of the rat's stomach which he attributed to infestation with a nematode, Spiroptera neoplastica (*Gongylonema neoplasticum*). Subsequent investigators discovered similar changes in the stomachs of rats free of parasites and expressed with increasing definiteness the conviction that they were dealing with the result of nutritional deficiencies. According to present-day standards, the white bread diet used by Fibiger [2] for his experimental animals was certainly inadequate in many respects.

Fibiger's original description was restricted to alterations in the rumen and succeeding experimenters continued to center their attention on the non-secretory portion of the stomach. From the standpoint of man and related species which do not have a rumen, experimental studies dealing with the secretory part of the rat's stomach are of wider interest and applicability than observations on the forestomach. Some investigators mentioned changes in the secretory epithelium when the rumen was affected, and in part described them. Howes and Vivier[3] gave a better account of these lesions than their predecessors, but they too recorded relatively few instances of abnormalities in the glandular mucosa in comparison with the occurrence of lesions in the forestomach. Howes and Vivier pointed out the similarity of the changes in the secretory and in the non-secretory epithelium.

It has been my experience that when the stomach is examined in the stretched-out condition, lesions are observed with greater regularity in the antrum than in the rumen, and within the glandular region there is a marked difference regarding frequency and type of lesion between antrum and fundus. The antrum is also distinguished from the other components of the stomach by the presence of lymphoid tissue. Histological study of the antral lesions by means of serial sections gives an insight into the nature of the pathological process in the mucosa.

This report is descriptive in character and deals with the pathological changes occurring in the different parts of the rat's stomach in nutritional gastritis. The dietary deficiencies concerned in the development of gastric lesions and details regarding nutritional effects will be discussed in another paper.

* Received for publication, March 15, 1941.

Methods

Seven hundred and twenty albino rats of both sexes were placed upon inadequate diets at 28 days of age. Various kinds of deficient diets were employed. One was the Pappenheimer and Larimore [4] diet consisting of a mixture of flour and salt. Another was the casein and starch diet used by Dalldorf and Kellogg,[5] Sure and Thatcher [6] and others. Several modifications with relation to protein, mineral salts and the B complex were made in the different diets. The feeding experiments were terminated at the end of 4 weeks when the animals were killed and their stomachs examined for lesions. As controls, 116 rats of both sexes and 56 days of age were examined after being kept for 4 weeks on a good stock diet.

The rats were killed with ether and complete autopsies were performed. The stomachs were removed immediately and opened along the greater curvature. After washing in normal saline solution, they were stretched by means of pins on a piece of cardboard and immersed in a 4 per cent solution of formaldehyde. After an interval of 24 hours, the specimens were examined for gross changes in the mucosa. Many lesions that were not seen in the fresh state became visible after formaldehyde fixation.

For histological examination, tissues were imbedded in paraffin and sections were stained with hematoxylin and eosin. In many instances serial sections of lesions were made. For control study, the entire antrum of a normal rat, 56 days old, was cut serially.

Gross and Microscopic Anatomy of the Stomachs of Rats Fed a Stock Diet

The rat's stomach is divided into two parts by a transverse, firm, white, raised ridge. The proximal portion is the rumen or forestomach which occupies about two-fifths of the total area of the stomach in the stretched condition. It has a thin, grayish white, translucent wall and a smooth, non-secretory internal surface. The esophageal orifice is just above the ridge on the lesser curvature. The portion between the ridge and the pylorus is the secretory or glandular area which in turn is subdivided into antrum and fundus. When the stomach is opened along the greater curvature, the antrum appears as a roughly semicircular area occupying the entire lesser curvature below the ridge. The antral wall is thin and translucent, and the lining is slightly roughened. The fundus includes the entire greater curvature below the ridge, except for a short distance above the pylorus. It has a thick, opaque wall and a velvety, furrowed surface. The border between antrum and fundus is demarcated by an abrupt elevation of the mucosa.

The rumen and dividing ridge are lined by a stratified squamous epithelium which is continuous with the epithelial lining of the esophagus. The mucosa is composed of a superficial thin layer of keratinized cells beneath which there is a layer of cells containing eleidin granules, and a deeper zone where the cells are nucleated. The mucosa is separated from an underlying, well developed muscularis mucosae by a

thin layer of connective tissue, which contains an occasional lymphocyte or eosinophilic polynuclear leukocyte. Another narrow band of loose connective tissue intervenes between the muscularis mucosae and the muscularis which is composed of inner circular and outer longitudinal fibers. At the limiting ridge, the epithelium is thickened and elevated, and at its junction with the glandular portion of the stomach there is a transition from the squamous to the columnar type of cell. Here, the submucosa is composed of dense connective tissue which is infiltrated by variable numbers of wandering cells and often contains glandular acini.

In about 15 per cent of all animals, one or more discrete, hard, white, raised spots measuring 1 to 2 mm. in diameter are found on the ridge or in the glandular region immediately adjacent to the ridge where the latter dips below the esophageal opening. Microscopically, such an area is a papillomatous projection of hyperplastic squamous epithelium continuous with the glandular mucosa and supported by a connective tissue stroma. All of the components of the gastric wall become thickened at the junction of the esophagus with the rumen.

The mucosa of the fundus consists of long, narrow, tubular glands lined by numerous large, round, prominent parietal cells and relatively inconspicuous chief cells. In the antrum, the glands are shorter and wider than in the fundus and are lined by columnar epithelium. Mitoses are not uncommon. The transition from fundic to antral mucosa is fairly abrupt and occupies an area of 300 to 500 μ. The glands are supported by a loose connective tissue stroma containing a few lymphocytes and polynuclear eosinophils. Brunner's glands extend into the antrum beneath the muscularis mucosae for a short distance above the pylorus. The muscularis mucosae and muscularis are continuous with the same structures in the rumen and are separated from one another by a layer of fine connective tissue.

Lymphoid tissue is found only in the antrum and exists in the form of large and small nodules and as diffuse lymphocytic infiltration. In control rats, large nodules occupy definite sites in the antrum. They are found in the midline just below the limiting ridge or along the fundic borders, more often on the left side than on the right. As a rule they exist singly. In rare instances two or even three may be present.

A large nodule appears as a circumscribed oval or round elevation of the mucosa measuring 2 to 4 mm. in diameter. It has a round, linear, or Y-shaped central depression. Microscopically, there is in the submucosa a sharply defined, nonencapsulated, densely packed collection of small lymphocytes. Germinal centers may be present. The overlying epithelium often shows at the fundic borders a transition from the antral to the fundic type of cell and is raised above the level of the neighboring mucosa. When a nodule exists just below the ridge, the overlying glands are sometimes hyperplastic. In the region of the central depression, the glands become short and shallow or are converted into a single flat layer of epithelium. The muscularis mucosae loses its continuity at the margins of the nodule which extends to the muscularis.

Large lymphoid nodules are of considerable interest and are being studied from two standpoints. One problem is concerned with whether they are normal structures corresponding to the lymphoid nodules in the small intestine [7, 8] or whether they are a manifestation of a nutritional deficiency in the stock diet. The other line of investigation deals with the incidence of nodules in gastritis. This phase of the study is complicated by the fact that it is difficult to distinguish, grossly, nodules from ulcerative lesions.

Small lymphoid nodules cannot be identified in the gross specimen. They vary considerably in different rats with regard to number, size and distribution. The nodules are oval in shape and measure from 30 by 80 μ to 120 by 180 μ. They are found most frequently near the midline or along the fundic borders, and consist

of localized, dense aggregates of small lymphocytes in the submucosa. Germinal centers are not present.

As a rule, diffuse lymphocytic infiltration in the submucosa is not encountered in control animals except in the prepyloric region; but in nutritional gastritis, infiltration is found frequently in association with mucosal lesions.

Mucosal Changes in Nutritional Gastritis

Antrum

The essential pathological change in the antrum consists of a break in the continuity of the surface lining of the mucosa in a region of epithelial hyperplasia. In the gross specimen there is a well defined, round, raised area measuring 2 to 4 mm. in diameter with a central depression which may be filled with a white or red plug. Instead of appearing as a small pit, in larger lesions the center may be an oval defect, or a linear or Y-shaped groove. There are marked variations in different animals with regard to the incidence and number of grossly visible ulcerations. The lesions are usually multiple and, in order of frequency, are found in the prepyloric region, on the lesser curvature and along the fundic borders. The distribution often corresponds to the course of the main gastric blood vessels.

The initial phases of the development of epithelial defects are not seen very often, even with the aid of serial sections. The earliest microscopic change is a minute break in the lining epithelium with an acute inflammatory reaction in the submucosa. Fibrinous edema, capillary dilatation and congestion, and leukocytes of the polynuclear variety appear in the loose connective tissue stroma. Polynuclear leukocytes are also found within the overlying epithelial cells, some of which show necrosis with a resultant break in continuity of the surface lining. Early changes are found in relatively normal mucosa, in areas of epithelial hyperplasia and at the sites of small and large lymphoid nodules.

Ultimately, one or more glands break down and the mucosal defect or ulcer involves the entire thickness of the mucosa and submucosa (Figs. 1 and 2). Ulcerations also occur in the epithelium covering large lymphoid nodules. Sometimes two lesions develop simultaneously and fuse to form a single large one. In some ulcers the remnants of necrotic glands are still visible in the base. In others, the defect extends to the muscularis mucosae which is infiltrated with polynuclear leukocytes. Occasionally the muscularis forms the floor. The crater is usually filled with an exudate composed of fibrin, leukocytic fragments, necrotic epithelium and red blood cells. Fresh hemorrhage may be present. The subjacent loose connective tissue is edematous and is invaded by numerous wandering cells, chiefly lymphocytes. The margins of the ulcer, which are abrupt and often undermined, are formed by hyperplastic glands whose lining cells frequently contain mitoses.

Areas of epithelial hyperplasia occur, particularly in the prepyloric region, without a demonstrable break in the mucosa. Serial sections of such areas often reveal central depressions containing short atypical glands which may represent the sites of healed ulcerations. Sometimes the elongated glands are tortuous and dilated, and the cytoplasm of the lining cells is vacuolated. The underlying loose connective tissue layers may be edematous and infiltrated by small lymphocytes.

When ulcerations are examined by the serial section method, it becomes evident that repair and injury go on simultaneously and that the pathological process is a continuous one. In many instances it can be shown in the same lesion that the mucosal defect is being covered with new epithelium in one place while necrosis and inflammation are active in another (Figs. 2 and 3). Various stages of injury and repair are also demonstrable in different lesions within the same antrum. At one site there may be fresh necrosis while at another there is no longer evidence of active breaking down of the mucosa and the defect is bridged by an uninterrupted layer of new epithelium. Later stages of repair are also observed where regenerating glands are filling in the craters of ulcers (Fig. 4). With complete glandular restoration, the site of a previous ulceration may not be identified or may appear as a depression in an area of hyperplasia.

However, healing does not always go on to complete regeneration of the mucosa. Instead, the newly formed epithelium may again break down and a lesion of chronic type develop as a result of recurrent injury and repair. Grossly, such an ulcer has a large crater and heaped-up, rounded edges. Microscopically, the floor of the defect is covered by a single layer of epithelium of recent origin, but the continuity of the new surface lining is broken by areas of fresh necrosis and hemorrhage. In the base of the ulcer there are a few short, atypical glands, young proliferating fibroblasts and capillaries. The crater contains fibrinopurulent exudate and blood clot. At the margins there are distorted hyperplastic glands and many of the epithelial lining cells are vacuolated. Figure 5 shows a large ulcer at the site of a large lymphoid nodule. The substance of the nodule is replaced to a great extent by young vascular connective tissue infiltrated by numerous eosinophils. The surface of the mucosal defect is incompletely covered by newly regenerated epithelium.

Rumen

The pathological changes in the mucosa of the rumen are essentially of the same nature as those in the antrum except that squamous instead of glandular epithelium is involved in the process. Lesions are found most frequently at the esophageal orifice and along the limiting ridge;

sometimes they are distributed over the entire surface of the rumen. Their number, size and shape are extremely variable and they tend to become confluent. As a rule, the lesions appear as discrete, irregularly round or oval, firm, mucosal elevations with central brownish depressions; or they may have a linear or stellate appearance. Sometimes cauliflower-like or papillomatous growths are present. The dividing ridge is frequently thickened and nodular, especially near the esophageal opening.

Microscopically, epithelial hyperplasia is particularly prominent and varies from a simple thickening, with rounded processes extending down to the muscularis mucosae, to long papillary outgrowths projecting from the surface. Mitoses are frequently seen. In the submucosa, leukocytic infiltration and dilatation of blood vessels often accompany marked hyperplasia. The muscularis mucosae and the muscularis may be hypertrophied.

Superficial necroses and deeper ulcerations occur in areas of epithelial hyperplasia. In a typical acute ulcer there is a complete break in continuity of the hyperplastic epithelium which forms the margins of a deep crater (Fig. 6). The floor of the defect is covered with necrotic epithelial cells and fragmented leukocytes. The subjacent submucosa and the layer of loose connective tissue between the muscularis mucosae and muscularis may show edema, capillary dilatation and cellular infiltration. The wandering cells are chiefly polynuclear eosinophils, but lymphocytes and mononuclear leukocytes are also present. Early healing is rarely seen but occasionally a single layer of young epithelial cells may be observed growing in from the edges and incompletely covering a mucosal defect.

Cyst formation also occurs in areas of epithelial hyperplasia. The cysts are irregularly shaped and extend through the muscularis mucosae down to the muscularis. Near the surface they are lined by hyperplastic squamous epithelium, but deeper there is a transition to low columnar epithelium. Numerous, smaller, glandlike structures lined by the same type of columnar epithelium accompany the larger cysts and invade the muscularis.

Fundus

Lesions in the fundus are uncommon even when the antrum and rumen show extensive pathological changes. The characteristic gross finding is mucosal hemorrhage in the form of multiple, pin-point petechiae or large, irregular, streaky blood clots. The bleeding is often so brisk that the entire gastro-intestinal tract is found at autopsy to be filled with fresh blood. Histologically, the hemorrhage is accompanied

by glandular necrosis, which is usually superficial but may involve the entire thickness of the mucosa. Epithelial hyperplasia is absent.

Gross and microscopical examination of the small and large intestine in a large number of the animals on deficient diets revealed no ulcerations or hyperplasia of the mucosa.

The following results were obtained in a group of 186 rats with gastric changes fed on a diet low in protein and deficient in the B complex: in 66, lesions appeared only in the antrum; in 30, abnormalities were present only in the rumen, and in 90, ulceration and hyperplasia occurred in both antrum and rumen. The fundus was the seat of hemorrhage in 19 animals but always in association with other lesions in the stomach.

Summary

In nutritional gastritis, the antrum and rumen may be involved separately or together. The antrum shows changes more often than the rumen. The fundus remains relatively free of lesions.

Lymphoid tissue is found only in the antrum. The significance of large lymphoid nodules is discussed.

The pathological changes in the mucosa of the antrum consist of ulcerations in areas of epithelial hyperplasia that tend to undergo spontaneous healing. Since various stages of injury and repair are found to exist simultaneously in the same lesion and in different lesions, it appears that the process is continuous. Recurrent necrosis and hemorrhage develop in healing defects and lead to chronic changes.

In the rumen, the mucosal abnormalities are essentially of the same nature as in the antrum except that hyperplasia is more striking in the rumen while healing of epithelial defects is more evident in the antrum.

The lesions in the fundus differ from those in the antrum and rumen and are essentially hemorrhagic erosions.

REFERENCES

1. Fibiger, Johannes. Untersuchungen über eine Nematode (Spiroptera sp. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwultstbildungen im Magen der Ratte hervorzurufen. *Ztschr. f. Krebsforsch.*, 1913, **13**, 217–280.

2. Fibiger, Johannes. Untersuchungen über das Spiropterakarzinom der Ratte und der Maus. *Ztschr. f. Krebsforsch.*, 1920, **17**, 1–79.

3. Howes, E. L., and Vivier, P. J. The relation of diet to the occurrence of gastric lesions in the rat. *Am. J. Path.*, 1936, **12**, 689–699.

4. Pappenheimer, A. W., and Larimore, L. D. The occurrence of gastric lesions in rats. Their relation to dietary deficiency and hair ingestion. *J. Exper. Med.*, 1924, **40**, 719–732.

5. Dalldorf, Gilbert, and Kellogg, Minerva. Incidence of gastric ulcer in albino rats fed diets deficient in vitamin B (B_1). *J. Exper. Med.*, 1932, **56**, 391–398.

6. Sure, Barnett, and Thatcher, H. S. Avitaminosis. XVI. Production of gastric ulcers in the albino rat as a result of specific influence of deficiency of vitamin B. *Arch. Path.*, 1933, **16**, 809–816.

7. Jordan, H. E. The significance of the lymphoid nodule. *Am. J. Anat.*, 1935, **57**, 1–37.

8. Hummel, K. P. The structure and development of the lymphatic tissue in the intestine of the albino rat. *Am. J. Anat.*, 1935, **57**, 351–383.

ADDITIONAL BIBLIOGRAPHY

Bisceglie, Vincenzo. Vitamine e tumori. I. L'alimentazione squilibrata in vitamine nei suoi effetti sugli innesti neoplastici. Introduzione e stato attuale del problema "alimentazione e tumori." *Tumori*, 1931, **5**, 145–166. Vitamine e tumori. II. Le proliferazioni epiteliali atipiche negli animali alimentati con dieta squilibrata in vitamine. *Ibid.*, 1931, **5**, 167–180.

Bonne, C. Cancer of the stomach of the wild rat and infection with a nematode worm, *Hepaticola gastrica* Baylis, 1925. *J. Trop. Med.*, 1926, **29**, 288–291.

Büchner, F.; Siebert, P., and Molloy, P. J. Über experimentell erzeugte akute peptische Geschwüre des Rattenvormagens. *Beitr. z. path. Anat. u. z. allg. Path.*, 1928–29, **81**, 391–425.

Bullock, F. D., and Rohdenburg, G. L. Experimental carcinomata of animals and their relation to true malignant tumors. *J. Cancer Research*, 1918, **3**, 227–273.

Buschke, A., and Langer, Erich. Tumorartige Schleimhautveränderungen im Vormagen der Ratten infolge von Teereinwirkung. *Ztschr. f. Krebsforsch.*, 1923, **21**, 1–10.

Buschke, A., and Peiser, Bruno. Epithelwucherungen am Vormagen der Ratten durch experimentelle Thalliumwirkung. *Ztschr. f. Krebsforsch.*, 1923, **21**, 11–18.

Carlson, A. Herbert Swift Carter Memorial Lecture, Columbia University, December 12, 1932.

Clemmesen, Svend. On arrest of growth and preserved capacity for growth in young rats suffering from partial vitamin B deficiency. *Acta path. et microbiol. Scandinav.*, 1933, **10**, 315–328.

Cramer, W. Papillomatosis in the forestomach of the rat and its bearing on the work of Fibiger. *Am. J. Cancer*, 1937, **31**, 537–555.

Drummond, J. C.; Baker, A. Z.; Wright, M. D.; Marrian, P. M., and Singer, E. M. The effects of life-long subsistence on diets providing suboptimal amounts of the "vitamin B complex." *J. Hygiene*, 1938, **38**, 356–373.

Findlay, G. M. Pellagra-like lesions associated with deficiency of vitamin B_2 in the rat. *J. Path. & Bact.*, 1928, **31**, 353–364.

Fujimaki, Yoshitomo. Formation of gastric carcinoma in albino rats fed on deficient diets. *J. Cancer Research*, 1926, **10**, 469–477.

Fujimaki, Y.; Kimura, J.; Wada, Y., and Shimada, S. Morphological changes of pavement epithelium of albino rats fed on vitamin A deficient diet. *Sei-I-Kwai M. J.*, 1927, **46**, 1–6.

Henning, N., and Norpoth, L. Untersuchungen über den Selbstschutz des Magens gegen Salzsäureverätzungen. *Arch. f. exper. Path. u. Pharmakol.*, 1932, **167**, 224–236.

Hoelzel, F., and Da Costa, E. Production of peptic ulcers in rats and mice by diets deficient in protein. *Am. J. Digest. Dis. & Nutrition*, 1937, **4**, 325–331.

Matzner, M. J.; Windwer, Charles, and Sobel, A. E. The role of protein in the prevention of experimental gastric ulcers. *Am. J. Digest. Dis. & Nutrition*, 1938, **5**, 36–38.

McCarrison, R. Experimental production of gastric ulcer in albino rats. *Indian J. M. Research*, 1931–32, **19**, 61–66.

Miller, S. P. Effects of inanition on the stomach and intestines of albino rats underfed from birth for various periods. *Arch. Path.*, 1927, **3**, 26–41.

Mitchell, H. S.; Bradshaw, P. J., and Carlson, E. R. Hair ball formation in rats in relation to food consistency. *Am. J. Physiol.*, 1924, **68**, 203–206.

Orten, A. U. Some observations on the effects of low levels of dietary protein. (Abstract.) *J. Nutrition*, 1939, suppl. **17**, 14.

Richards, M. B. The role of vitamin A in nutrition. *Brit. M. J.*, 1935, **1**, 99–102.

Santi, M. Ulcera gastrica e avitaminosi A. *Med. ital.*, 1931, **12**, 155–178.

Schiödt, E. Gastro-intestinal affections from vitamin deficiency. *Acta med. Scandinav.*, 1934–35, **84**, 456–495.

Sharpless, G. R. Malignant changes in the forestomach of rats. Related to low protein (casein) diet and prevented with cystine. *Ann. Surg.*, 1937, **106**, 562–567.

Sharpless, G. R. Prevention of hyperplasia in the forestomach epithelium of rats fed white flour. *J. Nutrition*, 1940, **19**, 31–37.

Sharpless, G. R. Choline and epithelial hyperplasia in the forestomach of rats. *Proc. Soc. Exper. Biol. & Med.*, 1940, **45**, 487–488.

Singer, Charles. The production of ulcer of the stomach in the rat. *Lancet*, 1913, **2**, 279–281.

Simpson, C. K. Observations upon gastritis. III. The gastric lesions of vitamin B_1 deficiency in the albino rat. *Guy's Hosp. Rep.*, 1936, **86**, 120–128.

DESCRIPTION OF PLATES

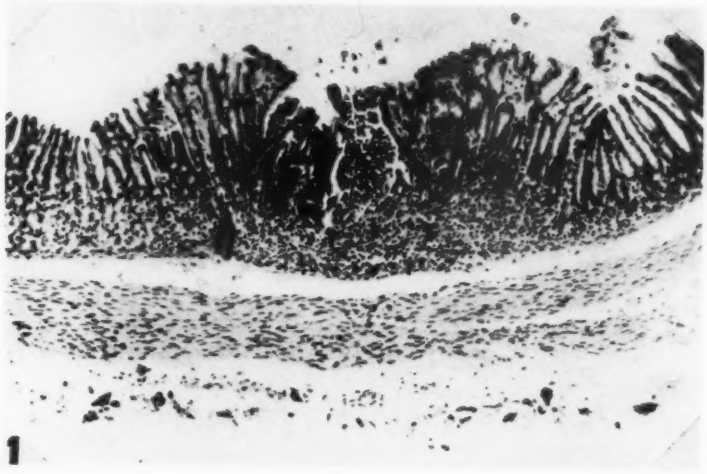
All illustrations were prepared from sections from the stomachs of rats on diets low in protein and deficient in the B complex. Hematoxylin and eosin stain.

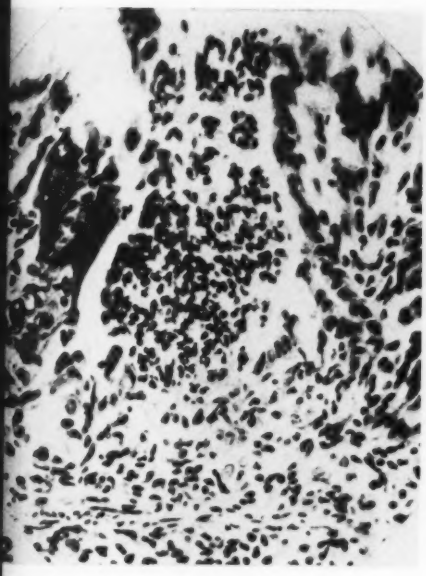
PLATE 9

FIG. 1. Antrum. An ulcer in an area of epithelial hyperplasia. ×110.

FIG. 2. Antrum. High power magnification of the ulcer in Figure 1. × 373.

FIG. 3. Another section of the same lesion showing repair. The base is covered with regenerated epithelium. × 373.


1


2

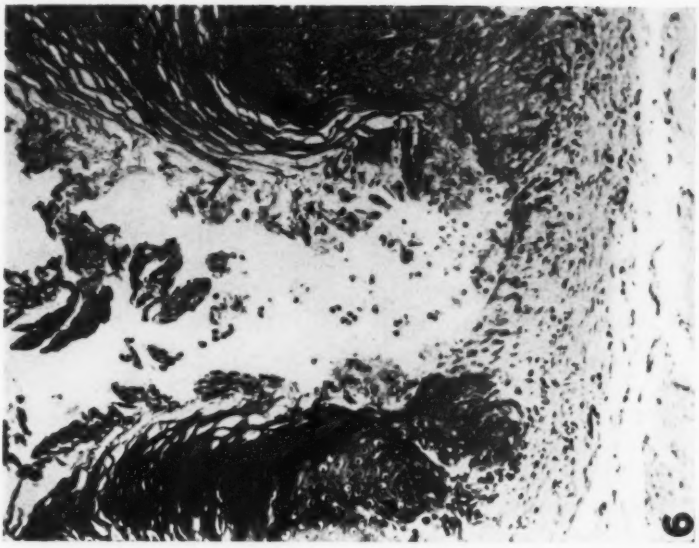
3

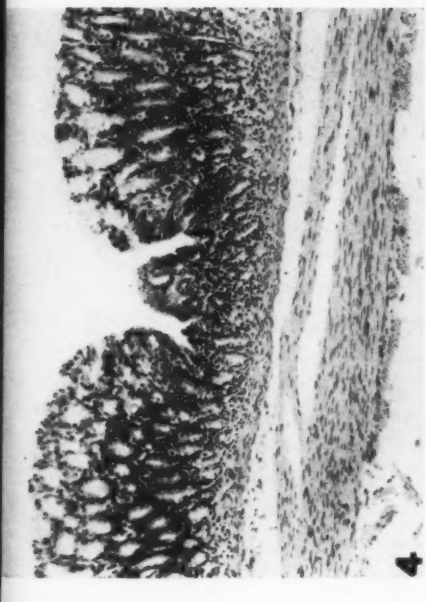
PLATE 10

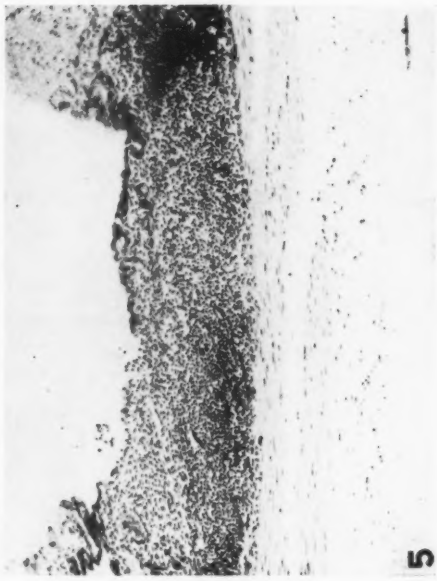
FIG. 4. Antrum. New gland formation in the base of a healing ulcer. × 95.

FIG. 5. A large ulcer with chronic changes at the site of a large lymphoid nodule. The base of the ulcer is partly covered with regenerating epithelium. × 95.

FIG. 6. Rumen. An ulcer in an area of epithelial hyperplasia. A single layer of proliferating epithelial cells is beginning to bridge the defect. × 230.


6


4


5

THE DISTRIBUTION OF INTIMAL ATHEROMATOUS LESIONS IN THE ARTERIES OF RABBITS ON HIGH CHOLESTEROL DIETS *

SIGMUND L. WILENS, M.D.

(From the Department of Pathology, New York University College of Medicine, New York, N.Y.)

Most of the numerous theories which have been advanced to account for the focal nature and usual distribution of early aortic atheromatous lesions are based on the assumption that the affected areas have been subjected to greater "stress and strain" in a selective fashion. This is said to lead to impairment of elasticity followed by fibrous overgrowth of the intima locally, or wrinkling with formation of connective tissue interstices (Duguid,[1] Moschcowitz[2]); swelling of the "ground substance" (Aschoff[3]); separation or actual intimal "herniation" (Krafka[4]); local anemia (Moon[5]); increased infiltration (Anitschkow[6]); or increased intimal vascularity and hemorrhage (Winternitz, Thomas and LeCompte[7]). Many of these theories are reviewed briefly by Krafka. All of them postulate a preliminary, preparatory alteration which induces the subsequent deposit of lipids.

In most instances, by the time fibrous proliferation has occurred in intimal plaques, lipids are present as well, so that it is impossible to be certain that the fibrous overgrowth is not entirely a secondary reaction to them. Even if lipid is not demonstrable, there is no assurance that it was not formerly present and has been reabsorbed. It is commonly believed that the lipidic streaks seen in intimas early in life are capable of reabsorption and disappearance. For example, the valvular deposits seen in infants are less frequently observed in older children (Aschoff,[3] Zinserling[8]) and this has been interpreted as indicating that such early deposits are not always permanent. If this is true, there is no reason why early accumulations of lipids elsewhere, such as in the vicinity of branch orifices, should not be equally reversible.

In a previous study of the postmortem elasticity of the adult human aorta, I[9] was able to confirm the observation of others (notably Duguid[1] and Moschcowitz[2]) that intimal plaques tend to occur in greatest number and size in those parts of the aorta which are least movable because of the vessel's attachments. It was also found that regardless of the amount of intimal plaque formation, such fixed areas of all aortas lose their extensibility equally and progressively with advancing age, and earlier and more rapidly than the more freely movable parts of the vessel.

* Received for publication, April 5, 1941.

From these findings, I advanced the hypothesis that intimal lipids, whether extracellular or contained within mobile phagocytes, tend to converge or migrate as a result of the pulse movements of the vessel towards points of least mobility, being impeded in their progress at points where firm scars have fused the media to the intima, obliterating potential tissue spaces. This might account for the accumulation which occurs, for example, at the scar of the obliterated opening of the ductus arteriosus. Similarly, progress might be arrested at points where arterial branches form sharp angles at their origins such as at the mouths of the intercostal arteries. The formation of discrete deposits at definite points in the aorta, according to this interpretation, would be the result of such intra-intimal convergence of lipids. In other words, regardless of the manner in which lipids may enter the intima—whether as a finely dispersed deposit directly from the lumen through the endothelium or from penetrating vasa vasora—their final resting places are not necessarily identical with the points of entrance.

To test this, it would be of interest to know if lipids deposited in the arteries of rabbits fed on high cholesterol diets undergo intramural adjustments in position. The basic disturbance which leads to the deposition of cholesterol compounds in the arteries of rabbits maintained on such diets may be radically different from that which obtains in man. Persistent hypercholesteremia and widespread lipidic deposits in extravascular sites, as has been frequently pointed out, are not associated with the human arterial lesions. Nevertheless, the arterial lesions of the rabbit are the closest approximation to the early human ones that have yet been achieved experimentally. Not only in their character but also in their distribution there are many striking similarities. This is especially true of the tendency of both to occur at the mouths of arterial branches. The only important discordant feature in distribution is the paucity of lesions in the abdominal portion of the rabbit's aorta as compared to the proximal portion. The reverse is the usual finding in man. However, it seems reasonable to assume that the conditions which lead to localization of lipids at the mouths of arterial branches in one species are also in effect in the other.

A number of studies have been reported on the relation of lipidic deposit to points of fixation in the arteries of rabbits fed high cholesterol diets. Anitschkow [10] observed that the intima, overlying the edges of medial lesions produced by injections of adrenalin, was frequently the seat of deposit of lipids following cholesterol feeding. Schilling [11] injured the rabbit's abdominal aorta under similar conditions. He failed to find cholesterol deposits at the actual site of injury but in one animal described a large deposit just proximal to the damaged area which he

believed was related not to the injury but to an arterial orifice in that region. However, large deposits of lipids at this distal point are unusual. In my opinion an alternative explanation for both this finding and for that of Anitschkow would be that lipid had collected at the margin of the injured areas but had failed to penetrate into the actual site because the reaction to the injury had caused firm union of the intima and media at this point.

Ssolowjew [12] made a more extensive study of this type. He injured arteries of rabbits by cautery before and during feeding with cholesterol. When the vessel was injured after hypercholesteremia had already been established, cholesterol was found in the actual field of injury and both intima and media were heavily impregnated. When the injured area was allowed to heal for 50 to 92 days before cholesterol feeding was instituted, the site of cauterization itself was devoid of lipids but a ring of cholesterol had accumulated around the margins in the intima. These findings I interpret as follows: When the vessel wall has been recently injured a local area of spasm, or at least of noncontractility, would serve as an immobilized zone and favor the collection of lipids from adjacent intimal tissue. The wall of the vessel itself being partly necrotic and inflamed, the lipids would permeate readily into the media through damaged elastic lamellae and smooth muscle cells, assuming that these, when viable and intact, offer an impassable barrier to intimal lipid. If, however, the injury had healed by fibrous tissue replacement before the blood cholesterol became increased, the site of previous damage would still be relatively non-contractile and immobilized but now lipids converging on the area would be unable to penetrate the dense fibrous tissue and would remain in the intima at its edges.

Harrison [13] caused medial calcification of the aortas of rabbits by administering large amounts of irradiated ergosterol prior to and during cholesterol feeding. He observed intimal deposits of lipids only in the uncalcified portions of the vessel and concluded that only the more mobile areas were susceptible to atheromatous formation. In his own descriptions and illustrations he shows that the intima overlying and adjacent to the margins of medial plaques of calcium contains the largest aggregates of lipids. This I again interpret as indicating that the lipids had penetrated the intima only at normal, uncalcified areas and had secondarily accumulated at the periphery of calcified, fixed areas. Failure to extend above the calcium masses would be due to the fibrosis, associated with the calcification, resulting in impenetrable fusion of the intima to the underlying tissue in these areas. This view is supported in another study of aortic calcification in hypervitaminosis

D by Duguid [14] in which secondary intimal changes of a proliferative type were described at the edges of the medial lesions. Later, Harrison [15] observed that rabbits' femoral arteries, rendered relatively relaxed, flaccid and presumably less mobile by previous sympathectomy, were more often and more severely involved by lipid deposits than the intact arteries of the opposite extremity.

It is difficult to devise a procedure which will immobilize portions of the arterial system without otherwise altering the same areas. If an artery is traumatized, its permeability to fluids and its adventitial blood supply are also changed. Even sympathectomy, as performed by Harrison,[15] while not directly injuring the femoral artery since the operative procedure involved removal of the second, third and fourth lumbar ganglia transperitoneally, may nevertheless lead to secondary structural changes dependent on denervation. Consequently, the results of all such experiments are open to more than one interpretation.

Lacking a method to immobilize a considerable portion of an artery without also injuring it, the next best procedure to test the hypothesis of intramural migration of lipids to areas of fixation, would be to devise a standard method of immobilizing a measured segment of an artery so that the attendant structural changes are approximately the same in all cases. Under such circumstances any differences in deposit of lipids at these sites would have to be accounted for on some other basis than the injury itself. The experimental data in this report record an attempt to accomplish this objective.

Materials and Methods

Nine adult rabbits, weighing about 2.5 Kg. each, were fed 1 gm. of cholesterol daily for a period of from 9 to 12 weeks according to the method recommended by Harrison.[15] The cholesterol was dissolved in 6 cc. of hot olive oil with enough bran added to absorb the oil.

Two days before cholesterol feeding was started, under nembutal anesthesia, cylindrical silver cuffs were placed on both common carotid and both femoral arteries. The cuffs were fashioned from sheet silver, 0.5 mm. in thickness, by cutting rectangular pieces 1.5 cm. in length and either 1.0 or 0.3 cm. wide. These were molded around cylindrical metal rods, of diameters from 0.1 to 0.4 cm., with a vise and pliers. Where the two edges came together to complete the hollow cylinder, the overlapping portions were reflected backward radially to the curved portion to form two flaps by which the cylinder could be opened or closed. These flaps were trimmed to 0.2 cm. in length and all edges were rounded by buffing. The cuff was opened sufficiently to admit the artery and closed afterwards with a heavy hemostat. The artery

was dissected free only at the actual point where the cuff was introduced. In every instance cuffs were selected with an internal diameter which was approximately equal to the external diameter of the artery so that the cuff fitted snugly, but without compression.

In five rabbits the cylindrical cuffs used were 1.0 cm. in length and either one or two of these were placed on each artery. In the other four rabbits, cuffs 0.3 cm. in length were used and from one to three were installed on each artery at varying distances. By measuring the distance of the cuffs from each other and from some fixed point in the surrounding tissue, it was determined that they were not displaced from their original location during the course of the experiment. Four other rabbits which were not fed cholesterol had similar cuffs placed on the common carotid and femoral arteries. These control rabbits were sacrificed and the vessels excised after 2, 6, 9 and 12 weeks. The reaction to the cuff itself was studied under a compound dissecting microscope and histologically.

At the conclusion of the experiment the cholesterol-fed animals were sacrificed by exsanguination and the arteries examined. Some were stained grossly with Sudan IV and fixed in a 10 per cent solution of formaldehyde to show the distribution of intimal lipid in relation to the sites of the cuffs. Others were examined unfixed and unstained in 0.9 per cent saline and subsequently fixed in Zenker's solution without addition of acetic acid. The entire vessel was embedded and sectioned serially. Every tenth section was stained alternately with hematoxylin and eosin and with combined Weigert and van Gieson stains.

All of the nine rabbits fed cholesterol exhibited the usual lipidic deposits but to a varying degree in the liver, adrenals, spleen, kidneys, aorta and pulmonary artery.

Results

Reaction of Arteries to Silver Cuffs

The silver cuffs became enveloped in a layer of fibrous tissue which was continuous with the adjacent adventitia at the ends of the cuffs. The thickness of this fibrous layer was considerably greater when the cuffs were 1.0 cm. than when they were 0.3 cm. long. In the case of the carotid arteries, this fibrous covering formed a distinct sheath or envelope which was only loosely attached to the surrounding fascia. The fibrous growth about the cuffs on the femoral arteries, however, extended more widely and was firmly united to adjacent structures, including neighboring veins, nerve trunks and muscle sheaths. When the fibrous sheaths were incised and cut away, the cuffs were found to be loosely held within them and could be readily slipped off of the

artery. Between the cuff and the underlying adventitia there was no union, although the adventitia was thickened by connective tissue proliferation which was continuous at the cuff's edges with the fibrous mass which covered the cuff externally. The surface of the silver cuffs was coated with fine brownish black precipitate which stained the adventitia and all the opposing surfaces of connective tissue as well. Sometimes this discoloration was visible through the intimal surface of the vessel. The arterial wall within the cuff was intact but thin. It was quite inelastic but still possessed considerable tensile strength. The intima within the 1.0 cm. cuffs often had a finely wrinkled or corrugated appearance, but that within the smaller 0.3 cm. cuffs was quite smooth.

Microscopically, the fibrous overgrowth in the adventitia consisted of irregularly disposed bands of collagen with collections of lymphocytes, macrophages and small capillaries. In arteries examined 2 weeks after application of the bands, cellular infiltration and capillary proliferation were more conspicuous and fibrous tissue formation less pronounced, but no suppuration or necrosis of tissue was observed. Extravasated erythrocytes and polymorphonuclear leukocytes were sometimes seen in small numbers. After 9 to 12 weeks the fibrous masses were largely avascular but still contained collections of lymphocytes and phagocytic mononuclear cells. The latter frequently contained a brownish, amorphous, granular pigment, the bulk of which failed to give the Prussian blue reaction for inorganic iron and probably was derived from the silver. Multinuclear foreign body giant cells were rarely encountered. The adventitia of the artery was thickened at the edges and within the cuffs. The media was thinned out to two-thirds or one-half of its normal thickness. This was more pronounced when 1.0 cm. cuffs had been used. The thinning began abruptly at the edges of the cuff and was quite uniform along its length. This narrowing was due chiefly to atrophy of smooth muscle, so that the elastic lamellae were crowded together with no intervening tissue except thin strands of fibrous tissue. Where this condensation was marked the individual elastic layers were barely discernible. In addition they frequently were ruptured and fragmented. The intima at the edges of the cuff was raised and thickened by a moundlike eminence of fibrous tissue, especially when 1.0 cm. cuffs had been used. This peripheral fibrous overgrowth of the intima was very scanty or completely absent when 0.3 cm. cuffs were employed. Within the field of the cuff the intima was as delicate as in the normal vessel. The fine corrugations seen grossly within 1.0 cm. cuffs were due to irregularities in the media of the vessel.

The changes observed in the vessel wall were always of the same type and approximately of the same extent, varying only in the latter respect with the size of the cuff used. Analogous alterations were found in the banded arteries of rabbits fed cholesterol.

Relation of Intimal Deposits of Lipids to the Sites of the Silver Cuffs

The amount of lipids found in the intimas of the arteries as a whole varied from none at all to numerous, heavy, almost confluent deposits. Similar unaccountable variations in lipids deposited in the femoral arteries of rabbits were observed by Harrison.[15] In general the femoral arteries had greater deposits than the carotids. It was at once apparent that when lipids were present at all they tended to accumulate in greatest amount in the vicinity of the cuffs. In some instances, when such deposits were scanty, the entire amount was limited to the regions of the cuff. When they were heavy, lipidic plaques were scattered throughout the vessel but were largest and most conglomerate where the silver bands had been placed.

The fatty deposits near the larger (1.0 cm.) cuffs were heaviest at the edges where they sometimes formed transverse linear bands 1 mm. or more wide, extending around the entire circumference. Occasionally, tail-like processes extended away from the edges of the cuff into the adjacent intima. Lipids were not found in any appreciable degree within the central portions of these larger cuffed areas. This was true even when lipidic deposits were abundant everywhere else in the vessel.

Lipids tended to overrun the entire zone of the smaller (0.3 cm.) cuffs, forming irregular plaques or complete annular bands, not limited merely to the edges. Often there was a clear zone up to 1.5 cm. in width on either side of the cuff but beyond this lipid-free area smaller, independent, spherical or oval collections of lipids were scattered at random. When two 0.3 cm. cuffs were situated within 1.0 cm. of each other on a carotid artery, no lipids whatever were found in the intervening zone even if the sites of both cuffs were heavily laden. Under similar circumstances in the femoral arteries the intervening zone often had large accumulations of lipids. This may be attributed to the fact that the more extensive periarterial fibrosis in the case of the latter artery tended to immobilize the entire area.

Histologically, the smaller lipidic aggregates consisted of clusters of spherical, swollen macrophages with foamy cytoplasm, with no other cells participating and without connective tissue proliferation. The thickening of the intima seemed to be due largely to the presence of these cells, although occasionally they were impacted against areas of fibrous tissue proliferation similar to those seen at the edges of 1.0

cm. cuffs in control animals which had not ingested cholesterol. With Sudan stains on frozen sections the bulk of the lipids was found within these phagocytes but there was always a small amount in fine-droplet form free in the intima. Crystals of free cholesterol were not seen. The entire lipidic mass appeared to be anisotropic in polarized light.

The larger collections of lipids appeared as loosely woven, pale-staining masses in sections stained by hematoxylin and eosin. There were scattered nuclei in them and a few delicate, pink-staining fibrils. Some of these nuclei were pyknotic; most of them appeared to be the nuclei of large mononuclear cells. The cell boundaries of foam cells were less readily distinguishable. In fat stains the lipids extended from the intact endothelial surface to the media and where the elastic fibers were ruptured some had found their way into this coat as well. A higher percentage of the lipids in these larger aggregates was extracellular. In sections stained for connective tissue and elastic tissue there was very little evidence of fibril formation in the plaques and no proliferation of elastic tissue filaments. For this reason the fibrous mounds which had formed at the edges of the larger cuffs, similar to those observed in control animals, were readily distinguishable. These mounds consisted of dense masses of collagen and contained fine elastic fibrils. Often the lipidic masses were piled up externally and laterally to these mounds as though they presented impermeable barriers to the passage of lipids toward the actual sites of the cuffs.

The most striking observation, however, was the finding that the cuffs in themselves did not influence the total amount of lipids deposited in any one artery. In some instances no lipids were found at any point in the artery even though the injury produced by the cuffs was just as marked as in others and even though the other arteries in the same rabbit showed the usual deposits. In other words, although the injured areas served as foci for harboring lipids, the amount of deposition was not proportional to the degree of injury, which was fairly constant in all cases; but varied rather with the amount of lipids deposited in the vessel as a whole. In Table I an attempt is made to demonstrate this point by summarizing the findings. All the data from both sizes of cuffs are grouped together and where a single artery was enclosed in more than one cuff the average amount per cuff is recorded. Three carotid and four femoral arteries had no detectable lipids at any point. There were three carotid arteries in which intimal lipids were not found in the mobile parts of the vessel but which contained slight to moderate amounts in the cuffed areas. On the other hand, all five carotid and four femoral arteries which had numerous plaques throughout the mobile portions had very extensive deposits in the regions of the cuffs.

Discussion

These results do not support the contention that lipids accumulate in the intima at points of relative immobilization because structural changes attendant on fixation may lead to increased local permeability or special affinity for lipid. Conditions favorable to cholesterol deposition were present in all nine rabbits and such deposition did occur somewhere in the arterial system in every instance. Yet in 7 of 36 arteries no lipids penetrated at sites which were invested by silver bands. In the other 29 arteries these banded areas harbored more

Table I

Relation of Intimal Deposition of Lipids at Sites of Silver Cuffs to Amount of Lipids in Other Parts of the Same Artery

	Lipids in non-cuffed areas	Lipids in and adjacent to cuffed areas					Number of arteries
		None	Slight	Moderate	Marked	Very marked	
Carotid artery	None	3	2	1	—	—	6
	Slight	—	2	1	—	—	3
	Moderate	—	—	—	2	2	4
	Marked	—	—	—	—	5	5
Femoral artery	None	4	—	—	—	—	4
	Slight	—	—	—	3	2	5
	Moderate	—	—	—	1	4	5
	Marked	—	—	—	—	4	4
	Totals	7	4	2	6	17	36

lipids than the neighboring portions of the vessels. The morphological changes in the media and externa of the vessels were not appreciably different in these two groups. If some special type of injury alone is the deciding factor in controlling deposition of lipids, then its nature is not apparent histologically.

On the other hand, the findings do favor, without furnishing direct proof, the hypothesis that relatively fixed areas of the artery serve as traps which collect both intracellular and extracellular lipidic droplets as they drift through tissue spaces of the intima. The factor which determined the amount of lipids found at the sites of the silver cuffs appeared to be the amount found in the vessel as a whole. That the localization of lipids in the cuffed regions was not purely fortuitous is indicated by the fact that more lipids were invariably located here than at any other one point in the same vessel. Other findings which favor this interpretation are the localization of lipidic masses external to, but in immediate contact with, the zones of fibrous intimal proliferation caused by the cuffs themselves, and the occurrence of lipid-free zones in the intima on either side of regions which were immobilized by the cuffs. This latter finding suggests that lipids in the immediate vicinity had drained to the banded areas of the vessel.

If it could be proved that the formation of circumscribed masses of

lipids in the intima is the result of aggregation of a finely scattered deposit due to shifting of the material within potential intimal spaces, a number of apparently unrelated and even contradictory observations on the pathogenesis of human lesions could be coördinated into a single thesis. For example, it would be possible to account for the lipidic intimal streaks unassociated with fibrous proliferation as an early phase in the formation of discrete plaques of more complex structure. These have frequently been interpreted as having an entirely independent origin from so-called true atheromatous plaques.[1]

It would also be possible to postulate that diffuse intimal proliferation is not necessarily the inevitable result of ageing but is rather due to the repeated passage of lipids through intimal tissue. In rats which were allowed to live their entire life span of about 3 years, no equivalent thickening of the intima comparable to that of man was observed.[16] The aortic media of these animals underwent changes analogous to those seen in aged human aortas. It became thicker and the elastic lamellae lost their undulant character. The value of such a comparison is limited by the marked discrepancy in life span of the two species, but it is worthy of note that intimal deposits of lipids were never observed in the arteries of senile rats.

More important, the proof of intramural convergence of lipids to fixed areas would obviate the present necessity of assigning a dual origin for the formation of these lesions as does the "combination theory" advocated by Anitschkow.[6] On the one hand it is necessary to assume that local alterations precede and prepare the site for lipidic infiltration and on the other that changes occur in the circulating blood which permit the escape of cholesterol esters and other lipids into the arterial wall. It has been exactly on the importance of the relative rôles of these two processes that much controversy has developed in recent years.

By applying the theory of movement of lipids advanced in this report, it is possible to account for most of the vagaries in distribution of the early and late aortic lesions and of those seen in young and old individuals. In infancy and childhood, all parts of the aorta being equally and quite freely movable and scarred areas being absent, there is little tendency for lipids to pool in definite spots and when present they appear as irregular, often diffuse streaks in the proximal portions of the vessel. Only at valvular attachments or at the closed aperture of the ductus arteriosus do such migrating masses come to rest. Later in early adult life when the posterior thoracic and lower abdominal portions of the vessel already exhibit a measurable reduction in post-mortem elasticity[9] and anchorage to the adjacent bony structures is more rigid, such lipidic streaks tend to converge on these areas and to

form more permanent plaques with secondary changes in the tissues about them. With advancing age the movement of lipids is impeded further by plaques which have already formed and become invested by hyalinized connective tissue, and by the loss of elasticity and mobility in the remaining portions of the vessel. Eventually, if lipids continue to be deposited, the original pattern of selective localization is largely lost and the plaques are scattered more or less at random. Intercurrent syphilitic aortitis will cause many points of intimal fusion and hyalinization as well as diminished elasticity in the proximal part of the aorta and thus dislocate the movement of lipids.

Summary

Portions of femoral and carotid arteries of nine rabbits were rendered relatively immobile by being invested *in situ* by cylindrical silver cuffs of two uniform sizes. These animals were each fed 1 gm. of cholesterol daily. The changes induced in the arterial walls and adjacent tissue by cuffs alone were studied in four other rabbits. These proved to be fairly constant in type and extent. Briefly, they consisted of adventitial thickening and fibrosis, medial thinning due to atrophy of smooth muscle and condensation and fragmentation of elastic fibers. The intima remained thin and delicate save at the edges of the larger (1.0 cm. wide) cuffs where small mounds of fibrous tissue formed.

Cholesterol feeding usually led to selective localization of lipids in the intima of the arteries at the region of the cuffs. The amount of lipids present could not be correlated with the degree of injury produced by the cuffs. In 7 out of 36 arteries no lipids were found in the vicinity of the cuffs even though the injury to the vessel walls was as extensive as in the 29 others in which lipids in variable amounts were found.

The amount of lipids found at the site of the cuffs invariably exceeded that found at any other point in the vessel, indicating that conditions at these areas were favorable for their accumulation. The amount of lipids at the sites of the cuffs was directly proportional to the amount in the vessel as a whole.

These findings are interpreted as indicating that the injury itself was not the decisive factor in the deposition of lipids in the immobilized areas. Another and more plausible explanation is advanced; namely, that the immobilized areas served as foci at which the movement of lipids in intimal spaces, impelled by pulsations of the vessel wall, was arrested. The hypothesis is advanced that the points at which lipids accumulate permanently under natural conditions in arterial walls are not necessarily the same points at which they enter the intima but rather that they represent areas in which lipids are aggregated by a process of intramural migration.

REFERENCES

1. Duguid, J. B. Atheroma of the aorta. *J. Path & Bact.*, 1926, **29**, 371–387.
2. Moschcowitz, Eli. The cause of arteriosclerosis. *Am. J. M. Sc.*, 1929, **178**, 244–267.
3. Aschoff, Ludwig. Lectures on Pathology. Paul B. Hoeber, Inc., New York, 1924, pp. 131–153.
4. Krafka, Joseph, Jr. The mechanical factors in arteriosclerosis. *Arch. Path.*, 1937, **23**, 1–19.
5. Moon, V. H. Atheromatous degeneration of the arterial wall. The result of a hydrodynamic mechanism. *Arch. Path.*, 1927, **3**, 404–408.
6. Anitschkow, N. Experimental Arteriosclerosis in Animals. In: Cowdry, E. V. Arteriosclerosis. The Macmillan Co., New York, 1933, pp. 308–315.
7. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M. Studies in the pathology of vascular disease. *Am. Heart J.*, 1937, **14**, 399–404.
8. Zinserling, W. D. Untersuchungen über Atherosklerose. 1. Über die Aortaverfettung bei Kindern. *Virchows Arch. f. path. Anat.*, 1925, **255**, 677–705.
9. Wilens, S. L. The postmortem elasticity of the adult human aorta. Its relation to age and to the distribution of intimal atheromas. *Am. J. Path.*, 1937, **13**, 811–834.
10. Anitschkow, N. Einige Ergebnisse der experimentellen Atherosklerose-forschung. *Verhandl. d. deutsch. path. Gesellsch.*, 1925, **20**, 149–154.
11. Schilling. Experimentelle Erzeugung von Intimahyperplasien. *Verhandl. d. deutsch. path. Gesellsch.*, 1925, **20**, 154–165.
12. Ssolowjew, A. Experimentelle Untersuchungen über die Bedeutung von lokaler Schädigung für die Lipoidablagerung in der Arterienwand. *Ztschr. f. d. ges. exper. Med.*, 1929–30, **69**, 94–104.
13. Harrison, C. V. Experimental arterial disease produced by cholesterol and vitamin D. *J. Path. & Bact.*, 1933, **36**, 447–453.
14. Duguid, J. B. Vitamin D sclerosis in the rat's aorta. *J. Path. & Bact.*, 1930, **33**, 697–711.
15. Harrison, C. V. The effect of sympathectomy on the development of experimental arterial disease. *J. Path. & Bact.*, 1939, **48**, 353–360.
16. Wilens, S. L., and Sproul, E. E. Spontaneous cardiovascular disease in the rat. II. Lesions of the vascular system. *Am. J. Path.*, 1938, **14**, 201–216.

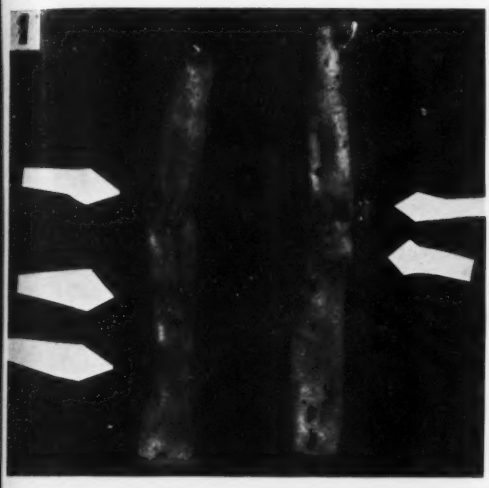
DESCRIPTION OF PLATES

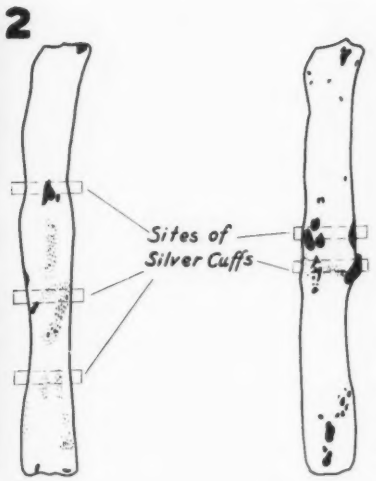
PLATE 11

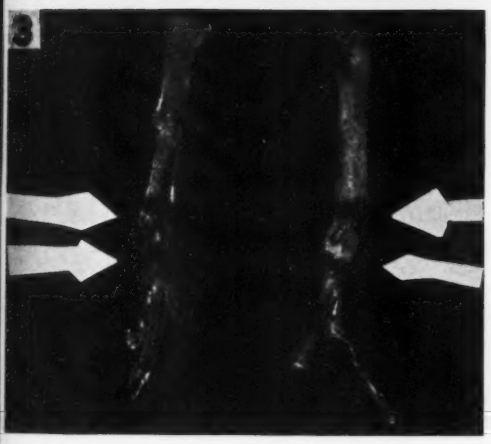
FIGS. 1 and 2. A photograph and drawing of two carotid arteries showing the distribution of intimal lipids in relation to the sites of the silver cuffs. The artery on the left shows very little lipid and most of it is in the vicinity of the cuffs. The one on the right shows more extensive deposits, most of which are in the immobilized area. Adjacent to this area there is relatively little lipidic deposit but at a distance the plaques are again numerous.

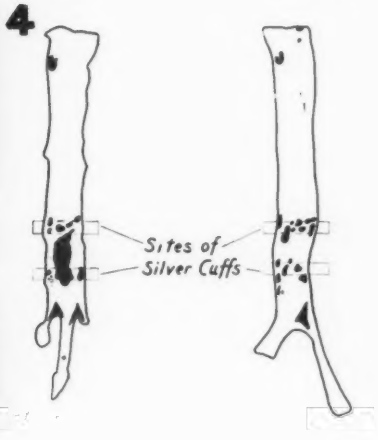
FIGS. 3 and 4. A photograph and drawing showing intimal lipids in two femoral arteries. The bulk of lipidic deposit is contained in and between the sites of the silver cuffs.

FIG. 5. Preparation stained by Weigert's and van Gieson's stains showing entire span occupied by a silver cuff, 1.0 cm. wide, on femoral artery. The adventitial fibrous tissue has been dissected away except at the edges where it enclosed the cuff. The media has been greatly thinned out and its elastic lamellae pushed together. The intima in the field of the cuff is thin but at its edges there are two moundlike elevations in which an abundance of lipid was easily demonstrable. × 8.


1


2
Sites of
Silver Cuffs


3


4
Sites of
Silver Cuffs

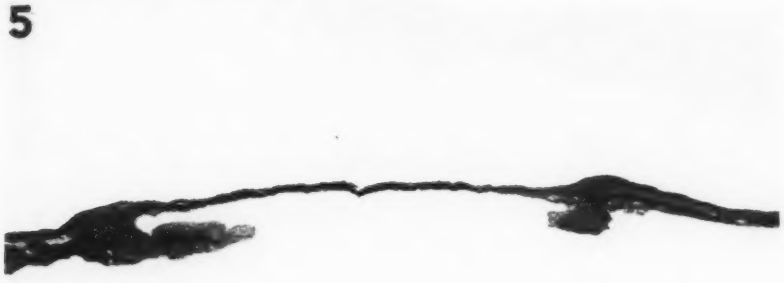
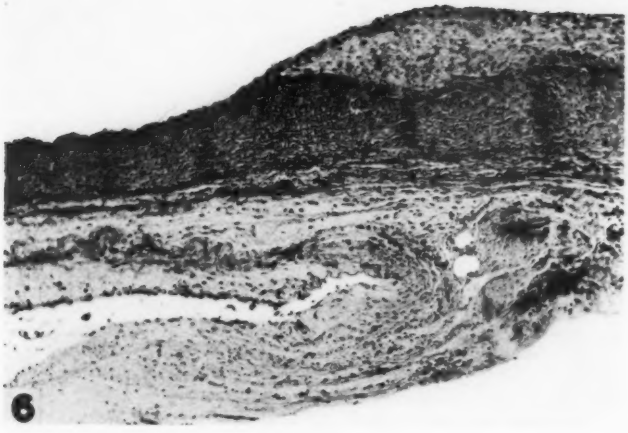

5

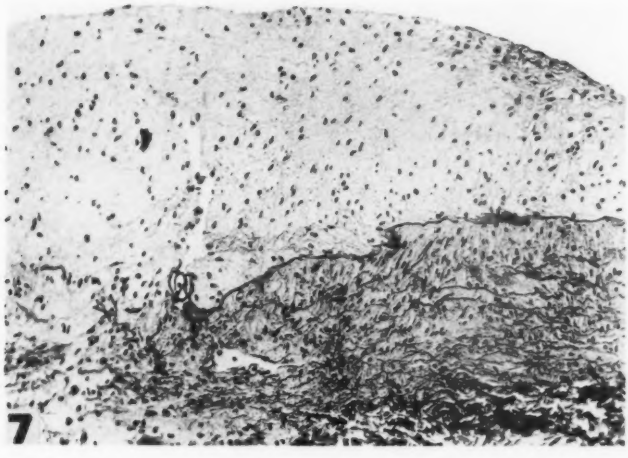
PLATE 12

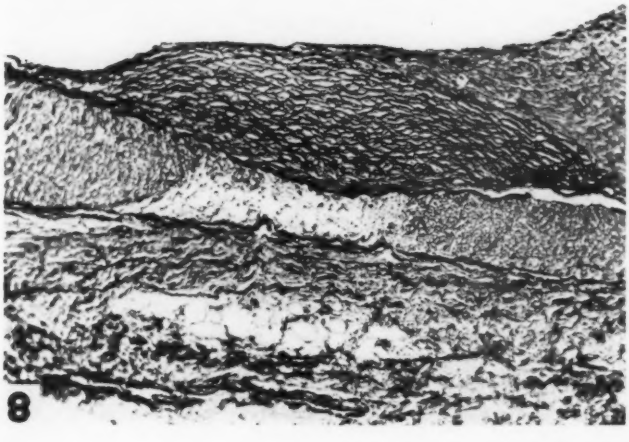
FIG. 6. Section of a carotid artery stained by hematoxylin and eosin, showing a cellular and partly fibrous intimal plaque situated at the edge of a cuffed area and extending beyond it. Lipids were demonstrated in this plaque by staining frozen sections with Sudan stain. The exact position of the edge of the silver cuff is indicated in the fibrous adventitia by the horizontal slit in which the cuff was lodged. × 79.

FIG. 7. A higher magnification of an intimal plaque situated over the edge of a silver cuff. The pale-staining and loosely woven texture of the plaque is due to the presence of extracellular lipidic droplets. Foam cells are also present but do not show clearly. The media at the edge of the cuff is markedly disrupted. × 103.

FIG. 8. Section of a femoral artery, prepared with Weigert's and van Gieson's stains, at the edge of a silver cuff, 1.0 cm. wide. The media is sharply narrowed at this point and over it is a crescent-shaped mound of connective tissue in the intima, containing many delicate elastic fibrils. Similar areas of intimal hyperplasia were observed in this region in rabbits which were not fed cholesterol. Just external to this intimal mound and piled up against one side of it is the edge of a lipid-containing atheromatous plaque. × 106.


6


7


8

THE PATHOLOGY OF CHASTEK PARALYSIS IN FOXES *

A Counterpart of Wernicke's Hemorrhagic Polioencephalitis of Man

C. A. Evans, M.D., W. E. Carlson, B.A., and R. G. Green, M.D.
(From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.)

A specific dietary disease of silver foxes, which we have recognized since 1932 as one of the important problems of the fur industry, is characterized pathologically by lesions similar to those of Wernicke's disease of man. The purpose of this paper is to describe the pathologic picture of the disease as it occurs in foxes and to clarify the relationship it bears to Wernicke's disease. The epidemiologic and nutritional aspects are described in greater detail elsewhere.[1]

We first observed this disease among foxes in February, 1932, when it appeared among the animals on the Chastek fur farm in Minnesota, and, during a period of 1 month, killed one-third of the entire ranch population of more than 200 foxes. In 1936, one of us (Green [2]) described the disease as a clinical entity and pointed out its occurrence as related to the feeding of fresh fish. A year later we [3,4] reported its experimental reproduction and the cause as a vitamin B deficiency. Carlström and Jonsson [5] reported the disease in Sweden in 1938 and obtained recovery of one fox by the injection of pure vitamin B_1. More recently, Ender and Helgebostad [6] have confirmed our reproduction of this disease experimentally, describing the condition as experimental beriberi. We have studied more than ten outbreaks in five states since 1932 and have shown that the natural outbreaks of the disease are usually induced by feeding foxes and minks fresh fish and that the disease is purely a vitamin B_1 deficiency. In a recent experiment a group of 12 foxes given a diet including carp as 20 per cent of the ration developed Chastek paralysis within 3 weeks, while a group of 16 foxes simultaneously receiving an identical ration plus 25 mg. of thiamin hydrochloride a day remained entirely well. The mechanism by which the presence of fish in the ration brings about deficiency of vitamin B_1 is not clear, but synthetic thiamin hydrochloride in large doses will prevent Chastek paralysis.

* From the coöperative investigations of the University of Minnesota, the Minnesota Department of Conservation, and the Fish and Wildlife Service, U. S. Department of the Interior, with the assistance of Fromm Laboratories, Grafton, Wis.

Presented at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1941.

Received for publication, May 8, 1941.

The usual history in a typical outbreak is as follows: Fish is introduced sometime in the fall or winter as from 10 to 50 per cent of the total ration. No untoward results are observed for a period of from 3 to 6 weeks. The foxes then go "off feed" and after a week or 10 days, individual animals have an abnormal gait; they may stagger or simply appear to be weak. Within 24 to 48 hours, such animals are usually seriously ill. Severe ataxia ensues, accompanied by a progressive spasticity which becomes so severe that finally the animals are unable to stand. Hyperesthesia may be marked, and the foxes usually moan constantly, as though in great pain, during the last 12 to 24 hours of life. The course of the disease is progressive; convulsions may occur, and animals usually die within 24 hours after developing ataxia and spasticity.

New cases appear in rapid succession. As already mentioned, we have observed the decimation of one-third of a ranch population of more than 200 foxes in a period of 1 month. Dramatic cessation of the outbreak can be brought about by removing fish from the ration and feeding a diet containing large proportions of liver. Animals in an early stage of the disease, or even those that show definite ataxia and early spasticity, can be cured by injecting thiamin parenterally and tube-feeding water, milk and liver juice.

Pathologic Findings in Organs Other Than the Brain

Although the most significant and diagnostic lesions of this disease are limited to the brain, many characteristic changes are found in other organs. The latter will be discussed first.

The nutrition of animals usually appears, at necropsy, to have been normal. This is somewhat surprising in view of the period of inappetence which precedes the onset of neurologic symptoms. Perhaps the small amount of food eaten by the foxes during this period is sufficient to keep them in good nutrition inasmuch as they are relatively inactive and maintain a low metabolic rate while sick with Chastek paralysis, as shown by subnormal body temperatures.

The lungs are negative except in an occasional animal in which there is a slight bronchopneumonia. The pleural, pericardial and peritoneal surfaces are normal. As a rule, no food is found in the stomach or intestines. Ulcers of the gastric mucosa are present more often than in foxes that die from other diseases. In different groups of foxes with Chastek paralysis, ulcers have been found in from 10 to 30 per cent of the animals. There is often a considerable amount of bile-covered mucus throughout the gastro-intestinal tract. Occasionally red blood, or black, tarry material is present in the intestines.

Degeneration of the liver is one of the most constant features of this

disease. The gross appearance of this organ, however, is variable, as it may be either obviously fatty or of a dark mahogany color. In either case the liver is usually soft and friable. Occasionally it has ruptured before death, causing hemorrhage into the peritoneal cavity. A moderate degree of icterus is seen in from 10 to 20 per cent of the foxes. In most animals the gallbladder is distended with bile. In about 5 per cent the liver is normal grossly and upon microscopic section is found to show only a small amount of fatty metamorphosis. Microscopic study of livers that were either fatty or of a mahogany color at necropsy shows a severe degree of lipoidosis to be present. In addition there may be necrosis of hepatic cord cells, as well as congestion and hemorrhage.

The spleen almost invariably is normal at necropsy, and microscopic sections show no change other than a moderate degree of hyperplasia of the lymph follicles. The kidneys are generally pale, and microscopically are found to have a few foci of lymphocytic infiltration among the tubules. There may also be some tubular degeneration. The urinary bladder, as a rule, is normal and may be empty or may contain a moderate amount of urine. The thyroid, adrenal, pancreas and salivary glands are usually normal.

Grossly, the heart may show cloudy swelling, and hemorrhages are present in about one-half the animals. Microscopically, the myocardium is sometimes normal but more often there are a few hemorrhages. Occasionally extensive pathologic alterations are found. The nucleoli of the myocardial cells may be so greatly enlarged as to resemble, superficially, intranuclear inclusions of a virus disease (Fig. 5). Areas of degeneration of myocardial fibers are seen associated with the hemorrhages. Proliferation of connective tissue, which extends outward from the perivascular areas to form a fairly dense meshwork among the degenerated cardiac muscle fibers, is also occasionally seen. The myocardial fibers in some cases appear to have a moderate intracellular edema, as evidenced by an increase in the size of the axial space. The combination of large nucleoli, focal necrosis of fibers, proliferation of connective tissue arising from the vascular tree and hemorrhages is diagnostic of Chastek paralysis, but one or more of these changes are lacking in a large proportion of cases. It appears that the neurologic manifestations of the disease usually kill the fox before cardiac lesions develop sufficiently to be diagnostic.

Pathologic Findings in the Brain

In the brain, the characteristic lesions of Wernicke's disease in man and of Chastek paralysis, which we believe to be the counterpart of that disease in foxes, are definitely focal in character and are highly characteristic in distribution and appearance. In both diseases strik-

ing proliferation of the endothelium and of the adventitia of small vessels occurs in focal areas. Irregular dilatation or varicose deformity of vessels is characteristic of both diseases. Numerous, small, parenchymal hemorrhages result from the degenerative vascular changes. A fully developed lesion appears as a hyperemic area in which there is an apparent marked increase in the number of small vessels, all of which are very cellular, and many of which are irregularly shaped (Figs. 2, 3 and 4). Alexander [7] demonstrated that the reported increase of vessels in the lesions of Wernicke's disease is only apparent and that with appropriate technics it may be shown that varicose dilatation and endothelial and adventitial proliferation have caused vessels that are not usually seen to become easily visible. Presumably the same is true in Chastek paralysis.

Degenerative parenchymatous changes occur in Chastek paralysis but are not so specific as the vascular changes. Nerve cells in the midst of a typical vascular lesion usually show degeneration or disappear altogether. As a rule, some proliferation of neuroglial elements is apparent. In an occasional animal, lesions are found in which fat-granule cells are exceedingly numerous, but more often these cells are relatively few. It is clear that the vascular changes do not represent a secondary phenomenon of the sort that follows ordinary tissue damage.

Although the histologic characteristics and general distribution of vascular lesions in the brains of foxes with Chastek paralysis have been familiar to us and have served as a basis for routine diagnosis during the past 5 years, we have only recently attempted to define precisely the location of these lesions, in order to make accurate comparisons with Wernicke's disease.

The sections used in studying the distribution of these vascular lesions were drawn from our regular files of animals submitted primarily for diagnosis. Our routine procedure in establishing a diagnosis includes obtaining eight or ten sections of the central nervous system from the following regions: cervical cord, medulla oblongata, mesencephalon, basal ganglia, cerebellum, and the olfactory, frontal, occipital and temporal lobes of the cerebrum. The exact location from which these sections are taken varies considerably in different animals.

Sections from 25 foxes were surveyed and more than 50 preparations were selected as showing typical lesions of Chastek paralysis. Of these foxes, 13 had died in ranch outbreaks and 12 had succumbed to experimentally-induced Chastek paralysis. These sections, in which well developed lesions were found, included 21 cut through the medulla, 12 through the cerebellum, 13 through the mesencephalon, 6 through the basal ganglia and 6 through the cerebral cortex. From these we have

obtained a composite picture of the pathology of Chastek paralysis. There are parts of the brain which are still unexplored, but the principal facts concerning the anatomic distribution of the characteristic vascular lesions are now available from the studies that have been completed.*

Wernicke,[8] in his original paper, commented on the fact that the lesions in human patients presented a bilateral symmetry with almost mathematical exactitude. No less striking is this symmetrical pairing of lesions in foxes with Chastek paralysis. Even before the microscopic pathology of this disease had been studied, we considered the gross appearance of bilaterally symmetrical hemorrhages in the brain stem highly characteristic of Chastek paralysis and quite different from the irregular distribution of the hemorrhages in other diseases, such as epizootic fox encephalitis. More recently it has become evident that the symmetry is also manifested by histologic changes. If the dorsal nucleus of the vagus nerve is involved on one side of the medulla, the corresponding nucleus on the opposite side will be involved by a lesion.

Another striking feature is that these lesions develop only in the gray substance of the brain. Never have we found a lesion in the midst of the pyramids or of the other portions of the brain which are devoid of nerve cells. This is an interesting fact when we remember that histologically the principal changes appear to be vascular.

The characteristic lesions of Chastek paralysis are limited not only to the gray substance of the brain but also, as a rule, to certain specific nerve centers. The term, paraventricular nuclei, has been used to describe, in a general way, the distribution of lesions in Wernicke's disease. This term applies equally well to Chastek paralysis in so far as the lesions in the dorsal nucleus of the vagus, the vestibular nuclei, the inferior colliculi, the nucleus of the third cranial nerve and the walls of the third ventricle are concerned. Typical lesions are also commonly found in the inferior olive and in the cortex of the splenial gyrus, both of which are a considerable distance from the ventricular system. On the other hand, lesions are often absent from the superior colliculi and the nucleus of the fourth cranial nerve, two structures which are well within the paraventricular gray matter.

There are certain nuclei that are practically never involved and others that show vascular lesions regularly. The presence or absence of lesions cannot be correlated solely with proximity to the ventricular system.

* M. A. Schadewald, formerly of the University of Minnesota and now at the University of West Virginia, identified, or verified the identity of, the nerve centers mentioned in this paper.

Distribution of the Characteristic Vascular Lesions in the Medulla Oblongata

In the medulla oblongata a lesion is usually present in the dorsal nucleus of the vagus. If it is severe, it may extend over the nucleus of the hypoglossal nerve, but the latter nucleus is seldom involved independently of the nucleus of the vagus. Typical lesions are frequently present in the medial vestibular nucleus, and often extend into the lateral, the superior, or the descending vestibular nuclei. It is not uncommon to see a marked vascular lesion in the medial vestibular nucleus, while one or more of the other vestibular nuclei are entirely normal. A lesion with very severe vascular changes is sometimes seen among the nerve cells lateral to the area ovalis of the corpus restiform and adjacent to the floor of the fourth ventricle.

Of four animals from which the selected sections passed through the upper end of the nucleus cuneatus and nucleus gracilis, three showed typical vascular lesions. Hemorrhages are common in the reticular formation but proliferative lesions do not occur there. In all cases, the pyramids were negative. Of six animals in which the sections included the inferior olive, three showed typical lesions. This is noteworthy, as already mentioned, in that it constitutes an exception to the general rule that lesions of this disease occur in the paraventricular area.

The bilateral symmetry of the lesions of Chastek paralysis is obvious in the medulla as well as in other parts of the brain. The few exceptions may be attributed to differences in degree of severity of lesions on the two sides. Occasionally, for example, lesions located primarily in the dorsal nuclei of the vagus may extend into the nucleus of the hypoglossal nerve on one side of the medulla but not on the other. It should be emphasized, however, that marked differences in severity of involvement on the two sides of the brain are the exception; it is the rule to find paired lesions of equal severity.

Distribution of the Characteristic Vascular Lesions in the Cerebellum

When present in the cerebellum, lesions are almost invariably located in folia close to the fourth ventricle. Apparently the vascular changes spread from the surface inward through the granular layer and into the molecular layer. Proliferation and irregular dilatation of vessels are most prominent in the granular layer, but hemorrhage is more extensive in the molecular layer and may force its way well into the white substance. Here, as elsewhere, the vascular pathology is found in the gray substance with only an occasional abnormal vessel extending into the adjacent medullary substance.

Distribution of the Characteristic Vascular Lesions in the Mesencephalon

The quadrigeminal plate is frequently involved and in all such cases lesions are present in the inferior colliculi. Occasionally the vascular changes extend anteriorly so as to form lesions in the superior colliculi, but more often sections through the superior colliculi are normal.

Ventral to the aqueduct, the nucleus of the third cranial nerve is the most common location of typical vascular lesions. The nucleus of the fourth nerve is seldom involved.

We have nearly always found the nucleus rubra to be normal, and have never seen a well developed lesion in the substantia nigra or basis pedunculi.

Distribution of the Characteristic Vascular Lesions in the Diencephalon

Many sections through the diencephalon of the selected 25 foxes were negative but some showed lesions in the thalamus and in the habenular nuclei. These lesions in some animals did not involve the parts closest to the third ventricle but were separated from the ventricular surface by a narrow zone of normal brain tissue.

Distribution of the Characteristic Vascular Lesions in the Cerebral Cortex

As a rule, routine sections from the olfactory, frontal, occipital and temporal lobes of the cerebrum are negative; but in a few of the foxes given special study, well marked vascular lesions associated with hemorrhages were present in the frontal and occipital lobes. These lesions were found most often in the vicinity of the splenial fissure, both in the frontal lobe near the anterior end of the fissure and in the occipital lobe close to its posterior end. In the occipital lobe there was, in some cases, bilateral selective involvement of the brain substance dorsal and posterior to the splenial fissure; *i.e.*, in the gyrus splenialis, the gyrus fornicatus on the opposite side of the fissure being normal or showing only moderate congestion. In the frontal lobe, the lesions were found on both sides of the splenial fissure. Many animals showed only congestion, with or without a mild endothelial proliferation, and some showed no lesions at all in this region. In an occasional animal, typical vascular lesions were found in foci adjacent to the fissura ectolateralis, the fissura suprasylvia posterior and the sulcus suprasylvius posterior.

In a few instances foci of varicose deformity and proliferation of small vessels involved the meninges as well as the subjacent cerebral substance. In all lesions of the cortex, the most severe vascular changes have been found in the superficial layers; *i.e.*, the molecular and outer

granular layers. Hemorrhages in many cases extended beyond the site of vascular damage, but the foci of degenerated and proliferated vessels were not found outside of the gray substance.

Discussion

A comparison of the disease which we designate as Chastek paralysis with that which Wernicke [8] described in 1881 as "die acute, hämorrhagische Poliencephalitis superior" shows some striking similarities.

Clinically, ataxia was noted by Wernicke, who stated: "Der Gang der Kranken wird taumelnd und zeigt eine Combination von Steifheit mit Ataxie, die am meisten an die Ataxie der Alcoholiker erinnert." [The walk of the patient becomes reeling and shows a combination of *stiffness with ataxia* (italics ours), which reminds one most strongly of the ataxia of alcoholic persons.] Progressive ataxia and spasticity are outstanding clinical features of Chastek paralysis.

Limitation of eye movements, noted by Wernicke, has not been studied in foxes, but there is ample basis in the pathology of the disease for some disturbance of ocular movements.

Principal emphasis was placed by Wernicke on the characteristic distribution of hemorrhagic lesions, as seen at autopsy. In summarizing briefly the findings in his second patient, he stated: "Am Gehirn derselbe Befund, wie im vorigen Falle. Die Blutungen gehen nirgends über die punktförmige Beschaffenheit hinaus und sind auf das centrale Höhlengrau des dritten und vierten Ventrikels sowie des Aquaeductus beschränkt." [The findings in the second patient were the same as in the first patient. The hemorrhages were confined to the central gray matter of the third and fourth ventricles and the aqueduct.] This statement and that already mentioned, "Die Veränderungen sind beiderseits mit fast mathematischer Genauigkeit gleich" [the lesions are equal with almost mathematical exactitude on the two sides of the brain], would both be valid in a general description of the characteristic lesions of Chastek paralysis of foxes (Fig. 1).

The reports of microscopic studies that Wernicke included in his original description were rather meager. However, they did point out that the hemorrhages and associated tissue reactions were confined to the nerve centers and "erstreckten sie sich nirgends auf die dem grauen Boden benachbarten Gebilde und Fasermassen" [never extended into the structures and nerve fiber masses in the vicinity of the gray matter]. This is a highly characteristic feature of Chastek paralysis, also. Never have we found a typical proliferative vascular lesion which developed outside of the gray matter.

In one case Wernicke mentioned that the region of the habenulae was

involved on both sides. This is a fairly common site of bilateral lesions in Chastek paralysis.

In his three cases, Wernicke did not find any marked changes in the vessel walls, nor did he observe any striking degree of endothelial proliferation. Subsequent workers have contributed much toward a more complete description of the vascular pathology in Wernicke's disease, and their findings coincide very closely with our observations in Chastek paralysis.

Focal lesions in which irregular dilatation of vessels and proliferation of endothelial and adventitial elements give the appearance of a marked increase in the number of small vessels, are known to characterize Wernicke's disease. These changes are identical with those of Chastek paralysis. In both diseases, hemorrhage is usually present in the midst of the vascular lesions.

Alexander, Pijoan and Myerson [9] have obtained evidence from their studies of human cases and their experimental observations on pigeons, as well as from the observations of Zimmerman [10] on dogs, of Prickett [11] on rats and of Pappenheimer and Goettsch [12] on chicks, that in these various species there can be produced a disease with distinctive pathologic features entirely analogous to those of Wernicke's disease in man. The combined evidence strongly supports Alexander's contention that Wernicke's disease of man and the parallel disorders of lower animals are due to a deficiency of vitamin B_1 existing in the presence of an adequate supply of other vitamins.

Chastek paralysis clearly belongs to the same group of diseases. It is the only one of them that occurs as a spontaneous disease in large animal populations. Dietary studies reported elsewhere would seem to prove independently of the pathologic evidence that Chastek paralysis is a B_1 avitaminosis.

A survey of clinical reports of patients with Wernicke's disease suggests that a disturbance of gastric function, perhaps achlorhydria, may be an important factor in that disease. One of the patients described in Wernicke's original report had severe gastritis as a result of sulfuric-acid poisoning; a considerable number of patients with carcinoma of the stomach have developed Wernicke's disease (Ecker and Woltman [13]); one case in which a patient with pernicious anemia had Wernicke's disease is mentioned by Alexander, Pijoan and Myerson [9]; and it is probable that many of those with chronic alcoholism have suffered from gastritis with a consequent disturbance of gastric secretion.

There can be no reasonable doubt that the feeding of fish causes Chastek paralysis, and it seems possible that some constituent of fish produces the B_1 avitaminosis by chemically inactivating thiamin in the

foxes' stomachs. If this occurs, achlorhydria may be a factor of considerable importance, inasmuch as thiamin is readily split in a neutral or alkaline solution but is stable in an acid medium. A study of gastric secretion in normal foxes and in foxes with Chastek paralysis is now in progress.

The selective localization of lesions of Chastek paralysis in certain nerve centers of the brain is probably of physiologic significance. It is known (Quastel [14]) that vitamin B_1 is necessary for the normal oxidation of glucose by brain tissue. In the absence of that vitamin there is an abnormal accumulation of pyruvic acid. Whether or not the lesions of Chastek paralysis and of Wernicke's disease represent a specific histologic response to an excess accumulation of pyruvic acid in the region of nerve cells affected by a lack of vitamin B_1 can be determined only by further experimentation. From the limitation of lesions to nerve centers and their selective distribution in the gray matter, it appears that some dysfunction of specific nerve cells resulting from a deficiency of vitamin B_1 is the basis for the observed vascular changes as well as for the parenchymatous degeneration.

Among nursing fox pups an acute form of Chastek paralysis, with a mortality rate of more than 30 per cent in 3 days, has been observed on two ranches. Fish or fish products constituted 16.5 per cent and 9.7 per cent of the rations on these ranches. At the time the pups were afflicted, the disease was just beginning to appear in adult animals.

Pathologically, the several adult animals that died in these outbreaks showed the usual lesions of Chastek paralysis. In the pups, however, the typical lesions were not present in the brain; no foci of vascular proliferation and varicose deformity were found, in spite of a careful search. Apparently the disease was so acute in nursing pups that death occurred before the characteristic histologic changes developed.

Summary

1. Chastek paralysis is an economically important, fatal, neurologic disease of foxes which is caused by a 10 per cent content, or more, of certain types of fresh whole fish in the ration. It can be prevented by adding large amounts of thiamin to the diet.

2. At autopsy the liver shows severe injury, and the heart occasionally contains characteristic lesions.

3. In the brain, bilaterally symmetrical lesions, limited to certain nerve centers predominantly in the paraventricular region, are diagnostic of Chastek paralysis. In these lesions there are irregular dilatation and striking proliferation of small vessels. The histologic changes and

the highly characteristic distribution of the lesions in this disease make Chastek paralysis the pathologic counterpart of Wernicke's hemorrhagic polioencephalitis of man.

REFERENCES

1. Green, R. G.; Carlson, W. E., and Evans, C. A. A deficiency disease of foxes produced by feeding fish. B_1 avitaminosis analogous to Wernicke's disease of man. *J. Nutrition,* 1941, **21,** 243–256.

2. Green, R. G. Chastek paralysis—a new disease of foxes. *Minnesota Wildlife Disease Investigation,* 1936, **2,** 106–107. Mimeographed and distributed by the United States Department of Agriculture.

3. Green, R. G. Chastek paralysis. *Minnesota Wildlife Disease Investigation,* 1937, **3,** 83–84. Mimeographed and distributed by the University of Minnesota and the Minnesota Department of Conservation.

4. Green, R. G.; Evans, C. A., and Carlson, W. E. A summary of Chastek paralysis studies. *Minnesota Wildlife Disease Investigation,* 1937, **3,** 173–177. Mimeographed and distributed by the University of Minnesota and the Minnesota Department of Conservation.

5. Carlström, Birger, and Jonsson, Gerhard. Ataxi, konvulsioner och acetonemi i samband med B-avitaminos. *Skandinavisk Veterinär-Tidskrift,* 1938, **28,** 144–148.

6. Ender, F., and Helgebostad, A. Eksperimentell beriberi hos sölvrev. *Skandinavisk Veterinär-Tidskrift,* 1939, **29,** 1232–1244.

7. Alexander, Leo. Wernicke's disease. Identity of lesions produced experimentally by B_1 avitaminosis in pigeons with hemorrhagic polioencephalitis occurring in chronic alcoholism in man. *Am. J. Path.,* 1940, **16,** 61–69.

8. Wernicke, Carl. Lehrbuch der Gehirnkrankheiten, für Aerzte und Studirende. Theodor Fischer, Kassel u. Berlin, 1881, **2,** 229–242.

9. Alexander, Leo; Pijoan, Michel, and Myerson, Abraham. Beri-beri and scurvy. An experimental study. *Tr. Am. Neurol. A.,* 1938, 135–139.

10. Zimmerman, H. M. The pathology of the nervous system in vitamin deficiencies. *Yale J. Biol. & Med.,* 1939–40, **12,** 23–28.

11. Prickett, C. O. The effect of a deficiency of vitamin B_1 upon the central and peripheral nervous systems of the rat. *Am. J. Physiol.,* 1934, **107,** 459–470.

12. Pappenheimer, A. M., and Goettsch, Marianne. A cerebellar disorder in chicks, apparently of nutritional origin. *J. Exper. Med.,* 1931, **53,** 11–26.

13. Ecker, A. D., and Woltman, H. W. Wernicke's disease: a neuro-ophthalmic syndrome of nutritional deficiency. *Proc. Staff Meet., Mayo Clin.,* 1939, **14,** 520–524.

14. Quastel, J. H. Respiration in the central nervous system. *Physiol. Rev.,* 1939, **19,** 135–183.

DESCRIPTION OF PLATE

PLATE 13

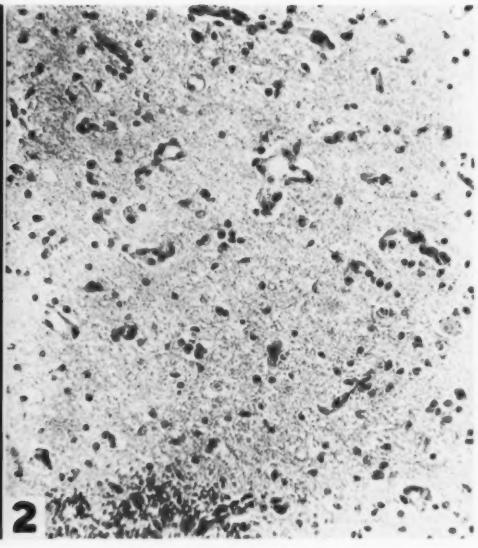
FIG. 1. Sections through the medulla oblongata and mesencephalon of three foxes showing the bilateral symmetry and specific localization of lesions in Chastek paralysis.

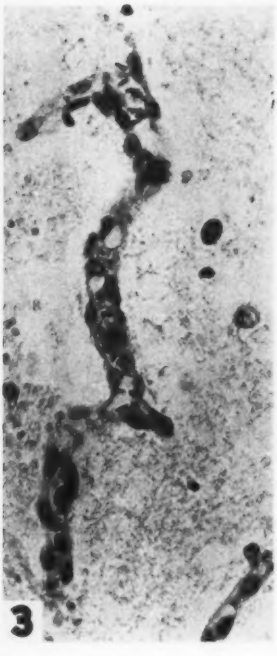
FIG. 2. Lesion in the quadrigeminal plate of fox no. R-25055. Endothelial swelling is marked in the small vessels. An area of hemorrhage is present near the lower border of the field. Hematoxylin and eosin stain. × 150.

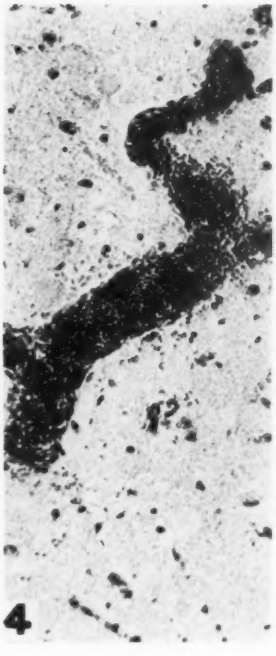
FIG. 3. Medulla oblongata, fox no. A-899, from a ranch outbreak. Marked endothelial swelling is present in a small vessel. Hematoxylin and eosin stain. × 900.

FIG. 4. Medulla oblongata, fox no. A-897, from a ranch outbreak. Blood vessel selected to show tortuosity and irregular dilatation. Hematoxylin and eosin stain. × 200.

FIG. 5. Heart, fox no. R-25053, from a ranch outbreak. Such large nucleoli are seen in the cardiac muscle of some cases of Chastek paralysis. They have not been found in foxes which have died from other causes. Hematoxylin and eosin stain. × 2200.







Evans, Carlson and Green Chastek Paralysis in Foxes

INTERCAPILLARY GLOMERULOSCLEROSIS *

ROBERT C. HORN, JR., M.D., and HANS SMETANA, M.D.
(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

In 1936, Kimmelstiel and Wilson [1] described eight instances of a renal lesion which consisted of hyaline thickening of the intercapillary connective tissue of the glomeruli, associated with arteriosclerosis. They termed the lesion "intercapillary glomerulosclerosis." Of their eight patients, seven gave a history of diabetes mellitus. Clinical data on the eighth were lacking. Anson [2] reported six additional cases in 1938. Newburger and Peters [3] tabulated the data of these reports, adding four autopsied cases of their own. According to them, the clinical syndrome associated with intercapillary glomerulosclerosis included diabetes mellitus, hypertension, widespread edema, albuminuria, hyposthenuria, nitrogen retention, hypo-albuminemia, retinal arteriosclerosis and, occasionally, heart failure and anemia. The syndrome as outlined by Porter and Walker [4] is essentially similar.

It is the purpose of this paper to point out that, although intercapillary glomerulosclerosis in its more advanced state is seen only in cases of diabetes, it is not of necessity associated with a particular clinical syndrome which includes diabetes mellitus.

The clinical histories, autopsy protocols and microscopic sections of all cases of diabetes and of arteriolar nephrosclerosis autopsied since 1909 at the Presbyterian Hospital, New York City, were reviewed. Satisfactory microscopic preparations were available from 144 cases in the former group and from 126 in the latter. Intercapillary glomerulosclerosis was noted in 33 of the 144 cases of diabetes mellitus, an incidence of 22.9 per cent (Fig. 1). Thirty-two instances, or 25.4 per cent, were found among the 126 cases of arteriolar nephrosclerosis without diabetes (Fig. 2).

The microscopic sections of the kidneys of 199 cases of glomerulonephritis autopsied during the same period were reviewed. Among these, 13 instances were found in which the lesions of many of the glomeruli were indistinguishable from those of intercapillary glomerulosclerosis (Fig. 3). Nine additional examples of this lesion were found among 81 cases of generalized arteriolar sclerosis without associated diabetes or renal disease (Fig. 4).

The incidence of intercapillary glomerulosclerosis in these various conditions is summarized in Table I.

* Received for publication, May 31, 1941.

One additional instance of early intercapillary glomerulosclerosis (autopsy no. 13545) was accidentally encountered in a female, 47 years old, with cirrhosis of the liver and Banti's syndrome who died following splenectomy. A few sclerotic arterioles were found in the kidney and pancreas of this case.

The age incidence of intercapillary glomerulosclerosis varied somewhat in the different groups. The average age at death of the patients with diabetes was 59.6 years; of those with arteriolar nephrosclerosis, 45.2 years; of those with generalized arteriolar sclerosis, 61.7 years, and of those with glomerulonephritis, 27 years. It is of interest that 82 per cent of the patients with both diabetes and intercapillary glomerulosclerosis were females, whereas only 54 per cent of the entire group of patients with diabetes were females. No such disproportion in sex distribution was noted in any of the other groups.

The pertinent clinical and pathologic data of these 88 cases of intercapillary glomerulosclerosis are summarized in Table II, according to the various categories of disease in which they were classified.

TABLE I

Incidence of Intercapillary Glomerulosclerosis

	Total no. cases	Intercapillary glomerulosclerosis	
		No. cases	Per cent
Diabetes mellitus	144	33	22.9
Arteriolar nephrosclerosis	126	32	25.4
Generalized arteriolar sclerosis (without diabetes or renal disease)	81	9	11.1
Glomerulonephritis	199	13	6.5

TABLE II

Intercapillary Glomerulosclerosis. Summary of Clinical and Pathologic Features

	Diabetes mellitus	Arteriolar nephro-sclerosis	Generalized arteriolar sclerosis	Glomerulo-nephritis	Total
Intercapillary glomerulosclerosis (number of cases)	33	32	9	13	88
	(per cent)	*(per cent)*	*(per cent)*	*(per cent)*	*(per cent)*
Albuminuria	83*	97	57	100	86
Hypertension (systolic pressure greater than 150 mm. Hg)	50*	94‡	67	77	71
Nitrogen retention	48†	93*	0*	92	70
Edema	30	47	22	38	36
Heart failure	42	56	22	15	41
Anemia (Hgb. less than 75%)	47*	78	0	77	59
Cardiac hypertrophy (greater than 400 gm.)	38‡	91	78	85	68
Arteriolar sclerosis, generalized	94	94	100	46	86
Arteriolar sclerosis, renal	100	100	100	100	100
Arteriosclerosis	94	88	78	31	80

* Data not recorded in 3 cases.
† Data not recorded in 12 cases.
‡ Data not recorded in 1 case.

Discussion

The results of serum protein determinations and of retinal examinations were available in too few cases to permit inclusion of these data. In our cases edema, except in glomerulonephritis, was generally dependent upon the presence of cardiac failure; and the anemia was usually secondary to renal decompensation. The clinical features which have been regarded as characteristic of intercapillary glomerulosclerosis are lacking in an appreciable proportion of these cases, among patients with diabetes as well as in the group as a whole. It is evident that the clinical signs and symptoms are not specific.

Herbut [5] and Allen [6] emphasized the specificity of the lesion of intercapillary glomerulosclerosis and suggested its use by the pathologist as a criterion for the morphological diagnosis of diabetes mellitus.* On the other hand, Kimmelstiel and Wilson,[1] in their original paper on glomerulosclerosis, called attention to the occurrence of identical lesions in the early stages of intercapillary glomerulonephritis unassociated with diabetes. Newburger and Peters [3] also studied a case of subacute glomerulonephritis without diabetes or generalized vascular disease in which the renal lesions were suggestive of intercapillary glomerulosclerosis. Our material includes 13 cases of glomerulonephritis, 10 chronic and 3 subacute, in which glomerulosclerosis was noted.

Our study indicates, further, that intercapillary glomerulosclerosis occurs not infrequently in association with conditions other than diabetes and glomerulonephritis, notably arteriolar sclerosis and arteriolar nephrosclerosis.

The case of cirrhosis of the liver, in which early lesions were seen associated only with mild arteriolar changes, is evidence that the study of routine sections of kidneys, with the possibility of glomerulosclerosis in mind, may lead to the discovery of other instances not associated with diabetes or renal disease.

It is important to note, however, that in every case where glomerulosclerosis was widespread or marked, review of the case record revealed a history of diabetes mellitus.

The frequency of arteriosclerosis and arteriolar sclerosis in patients with diabetes is well known. It has often been commented upon in association with intercapillary glomerulosclerosis as well. Nevertheless, the most striking finding emerging from our study is the extremely

* Since this paper was submitted for publication, the material upon which Dr. Allen's presentation was based has been published in the Archives of Pathology (So-called intercapillary glomerulosclerosis—a lesion associated with diabetes mellitus. Morphogenesis and significance. *Arch. Path.*, 1941, 32, 33–51.)

high incidence of arteriosclerosis and arteriolar sclerosis in these 88 cases of intercapillary glomerulosclerosis, and, in particular, the occurrence of sclerosis of renal arterioles in every instance. Newburger and Peters [3] suggested that the primary lesions were those of the arterioles, and that the diabetes and hypertension, as well as the renal changes, might be secondary.

Seventy of our cases of diabetes mellitus also showed generalized arteriolar sclerosis The diagnosis of intercapillary glomerulosclerosis was made in 31 of these, an incidence of 44.3 per cent. Furthermore, diabetes and nephrosclerosis occurred together in 22 instances and, of these, 13, or 59.1 per cent showed the lesions of intercapillary glomerulosclerosis. Anson [2] noted the presence of nephrosclerosis in all of his six cases.

Attempts to correlate these glomerular changes with hyalinization of the islets of Langerhans and with the specific therapy of diabetes were fruitless. Of 42 of our patients with diabetes dying before the introduction of insulin, 3 cases showed the lesions of intercapillary glomerulosclerosis.

Summary

The autopsy records of 550 patients with diabetes mellitus, arteriolar nephrosclerosis, generalized arteriolar sclerosis and glomerulonephritis have been reviewed. Among them, 87 instances of intercapillary glomerulosclerosis were found. In its advanced form, this condition was always associated with diabetes mellitus, and was present in 59.1 per cent of the cases of diabetes with arteriolar nephrosclerosis. Less severe degrees of glomerulosclerosis were found with equal frequency in cases of arteriolar nephrosclerosis without diabetes, and somewhat less often in cases of generalized arteriolar sclerosis and glomerulonephritis. One additional instance was encountered in a case of cirrhosis of the liver without diabetes, renal disease or generalized vascular disease. Sclerosis of renal arterioles has been found to be invariably associated with intercapillary glomerulosclerosis.

Note: We are indebted to Dr. Walter W. Palmer and to Dr. Allen O. Whipple for permission to use the clinical records of the patients included in this study.

References

1. Kimmelstiel, Paul, and Wilson, Clifford. Intercapillary lesions in the glomeruli of the kidney. *Am. J. Path.*, 1936, **12**, 83–97.

2. Anson, L. J. Intercapillary glomerulosclerosis. *South. M. J.*, 1938, **31**, 1272–1275.

3. Newburger, R. A., and Peters, J. P. Intercapillary glomerulosclerosis. A syndrome of diabetes, hypertension and albuminuria. *Arch. Int. Med.*, 1939, 64, 1252–1264.

4. Porter, W. B., and Walker, H. The clinical syndrome associated with intercapillary glomerulosclerosis. *J. A. M. A.*, 1941, 116, 459–464.

5. Herbut, P. A. Intercapillary glomerulosclerosis. *Arch. Path.*, 1941, 31, 501–507.

6. Allen, A. C. The morphogenesis and significance of intercapillary glomerulosclerosis. Paper read before the New York Pathological Society, New York City, January 23, 1941.

DESCRIPTION OF PLATE

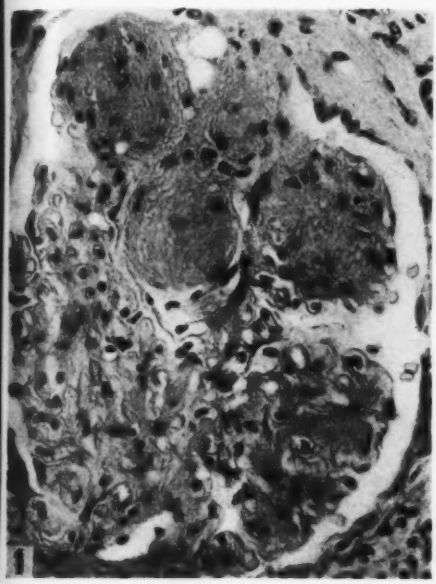
PLATE 14

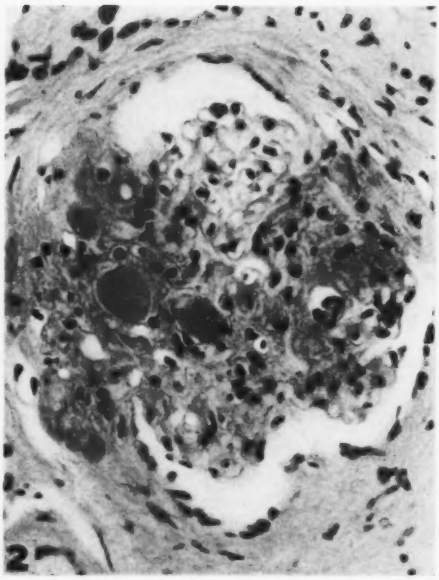
FIG. 1. Glomerulus from a case of diabetes mellitus (autopsy no. 13,286). Hematoxylin and eosin stain. × 394.

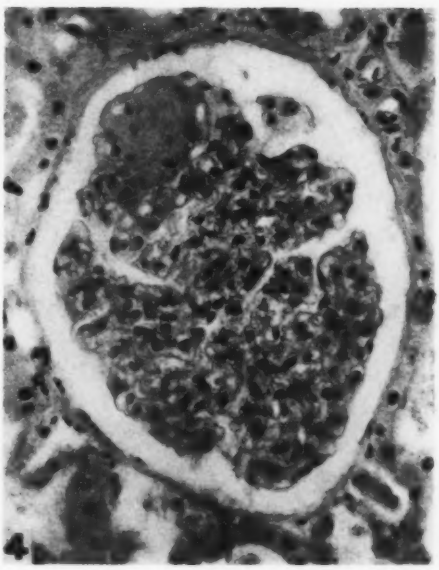
FIG. 2. Glomerulus from a case of arteriolar nephrosclerosis without associated diabetes (autopsy no. 11,757). Hematoxylin and eosin stain. × 394.

FIG. 3. Glomerulus from a case of subacute glomerulonephritis without associated diabetes (autopsy no. 11,459). Hematoxylin and eosin stain. × 394.

FIG. 4. Glomerulus from a case of generalized arteriolar sclerosis without associated diabetes or renal disease (autopsy no. 11,730). Hematoxylin and eosin stain. × 394.







Horn and Smetana

Intercapillary Glomerulosclerosis

GLOMERULONEPHRITIS IN PARTIALLY NEPHRECTOMIZED RATS*

Relation to Administration of Sulfapyridine

Paul Gross, M.D., Frank B. Cooper, D.Sc., and William A. Morningstar, B.S.
(From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh, Pa.)

Nephritis in rats is commonly exudative in character and is caused by ascending urinary infections or by bacteremia. However, in recent years experimental glomerular lesions, resembling those of human glomerulonephritis, have been produced in rats by the administration of nephrotoxic sera [1,2] or high protein diets.[2,3] A report by Jaffe,[4] in 1929, of an isolated instance of proliferative, non-exudative glomerulonephritis in a stock rat emphasizes the rarity of this lesion as a spontaneous disease.

The accidental finding of non-exudative glomerulonephritis in a rat given prolonged sulfapyridine treatment led to the present investigation of this lesion and its probable mechanism.

Method

Nineteen white rats, weighing 200 to 225 gm. each, were employed. Seven animals were subjected to a right partial nephrectomy involving the removal of less than one-half, probably only one-third, of the kidney. Eighteen days later a left nephrectomy was done on these rats and also on a group of five normal rats. Both groups were allowed to recuperate for 1 month. The remaining seven animals were not operated upon.

All animals were maintained on a standard diet, consisting of liberal amounts of dried bread, oats, carrots and Purina dog chow, prior to and during the experiment. The rats were given 50 mg. of sulfapyridine suspended in 0.5 cc. of 15 per cent gum acacia, daily, for the first 6 days, by stomach tube. For the next 3 weeks, except over the week-ends, the dosage was increased to 50 mg. twice daily; and then doubled again to 100 mg. twice daily, for the next 7 weeks.

At the beginning of the experiment and once weekly thereafter, each rat was subjected to cardiac puncture under ether narcosis during which 1.0 cc. of blood was removed for determinations of nonprotein nitrogen and of free and total sulfapyridine.

After 17 to 67 days of administration of the drug, about one-half of the animals died from cardiac tamponade or from anesthesia. The surviving rats were killed with ether 11 days after the last sulfapyridine treatment. Paraffin sections of the formaldehyde-fixed kidneys were

* Received for publication, May 5, 1941.

cut at 2 and 4 μ, and stained with hematoxylin and eosin and with azocarmine. Frozen sections were cut at 10 μ and stained with scarlet red.

A control series of ten rats was subjected to a right heminephrectomy during which somewhat more than one-half of the kidney was removed, followed, after 1 week, by removal of the left kidney. These animals received the same diet as those of the other series. They were given no drug, and were not subjected to any experimental procedure. Five deaths occurred: one on the second, two on the third, one on the sixth, and one on the twenty-eighth postoperative day. The five survivors were killed with ether 40 days after the last operation and sections of the kidneys were prepared as in the other series.

Results

Sulfapyridine-Treated Groups

Grossly, urolithiasis with hydronephrosis was encountered in two rats and dilated ureters in two others.

Histologically, the most advanced lesions consisted of focal proliferation of capsular epithelium with adhesions of the glomerular tuft to the capsule (Figs. 1 and 2). Many of the tufts were swollen and hyalinized and the subcapsular spaces were diminished. With the azocarmine stain, endothelial cells exhibited a homogeneous, dark blue cytoplasm which was continuous with the thickened basement membrane of the tuft capillaries. This thickening was most pronounced in the stalk of the glomerular tuft and frequently exhibited an arborescent pattern. The basement membrane of Bowman's capsule was unaltered except for reduplications of the membrane in the foci of glomerular adhesions (Fig. 2).

Many tufts showed foci of reduced cellularity and also foci of increased cellularity. In the latter, the nuclei appeared bunched and were frequently irregular in shape and hyperchromatic. These nuclei appeared to belong to proliferated epithelial and endothelial cells. The hyaline appearance of the tufts was caused mainly by swelling and hyalinization of these cells. The swollen endothelial cells frequently appeared to obstruct the lumina of capillaries. Such glomeruli were often bloodless.

Cloudy swelling of tubular epithelium associated with cellular disintegration and variable amounts of cellular débris in the lumina of the tubules were frequently found. Although these changes appeared to be of postmortem character in some animals, in others the alterations seemed to be antemortem. No fat could be demonstrated in the tubular epithelium or glomeruli.

Only four animals exhibited the histologic changes suggestive of urolithiasis medicamentosa.[5] These changes consisted of low-grade, subsiding, nonpurulent pyelonephritis with slight to moderate focal interstitial fibrosis and focal dilatation of convoluted tubules. In these regions finely divided fat droplets were demonstrable in the tubular epithelium.

Considerable scarring was encountered in a narrow zone upon the operated surface of heminephrectomized kidneys. Glomerular and tubular changes seen in this region were not considered pertinent.

The most widespread and advanced lesions were found in rats from which one and one-half kidneys had been removed, while animals which had not been operated upon exhibited the least involvement and relatively slight or early lesions. Unilaterally nephrectomized animals occupied an intermediate position relative to the amount and severity of renal involvement. Glomerular lesions were found in five of the seven rats operated upon bilaterally; three of the five operated upon unilaterally; and two of the seven animals not operated upon.

The weekly chemical determinations upon the blood showed occasional, sudden, transient increases of nonprotein nitrogen, up to 143 mg. per 100 cc., and of total sulfapyridine, up to 40.7 mg. per 100 cc., in a number of the animals. It was interesting to note that significant elevations in the blood sulfapyridine preceded elevations in the nonprotein nitrogen, and that after the latter had returned to normal the sulfapyridine level was still considerably elevated.

Non-Treated Control Group

All of the microscopic changes present in the drug-treated animals were also found in the non-treated group (Figs. 3 and 4). The severity and frequency of the lesions were no less than in the drug-treated rats which had subsisted with but one-half of one kidney. Only three of the ten control animals did not show glomerular lesions. Swelling and proliferation of the capsular epithelium were more pronounced; proliferation of the epithelium and endothelium of the tuft more frequent; and irregularity in the distribution of nuclei in the tufts more prominent in the control animals.

Scarring in the operated zone of the kidneys was pronounced. As in the drug-treated animals, focal pyelonephritis was encountered. This was generally more severe in the non-treated rats and was related, no doubt, to the lack of bacterial inhibition. The glomerular changes described in this paper were in regions uninvolved by either scarring or pyelonephritis. There were no vascular changes which could be incriminated in the production of the glomerulitis.

The kidneys of the control animals also showed considerable tubular dilatation associated with cloudy swelling of the lining epithelium.

Discussion

In previous experiments with rats [6] no significant glomerular changes were noted following the experimental production of urolithiasis medicamentosa with sulfapyridine. However, monkeys [7] treated with this drug were claimed to show a thickening of the basement membrane of the glomerular tufts.

The glomerular changes reported in the present experiment have not been seen previously with sulfapyridine or other sulfonamide drugs. The architectural alterations which heretofore have been observed with sulfapyridine, have been merely those incidental to urinary obstruction and to the secondary pyelonephritis. This fact, supported by the occurrence of identical glomerular changes in untreated rats, makes it very unlikely that sulfapyridine was directly responsible for the glomerular lesions. Furthermore, because such lesions were found predominantly in those rats from which one and one-half kidneys had been removed, it appears that glomerular damage was conditioned by a reduction in renal parenchyma. By causing urinary obstruction and thereby a further reduction in the functioning renal parenchyma, sulfapyridine apparently played a contributory rôle.

The fact that considerably more renal parenchyma was removed in the control rats than in the sulfapyridine-treated animals is offered as an explanation of the severity of the lesions in the untreated controls. Reduction in the amount of renal parenchyma was also an important consideration in the production of glomerular lesions by means of high-protein diets.[3] Unilateral nephrectomy was found to be a prerequisite in such experiments. The similarity of the lesions produced by two different methods suggests that the factor common to both methods—reduction in renal parenchyma with probable excessive work-demand placed upon remaining glomeruli—may be the immediate cause of the glomerular changes.

Although surgical reduction of renal parenchyma is not a factor in the glomerulitis produced by nephrotoxic sera, the similarity of the glomerular lesions to those produced by high-protein diets [3] and those described in the present experiments suggests the possibility that perhaps a functional reduction in renal parenchyma is a factor.

The irregular, abrupt, and often pronounced elevations in nonprotein nitrogen and in sulfapyridine content of the blood are adequately explained by urinary obstruction from acetylsulfapyridine uroliths. The return to normal values was caused by spontaneous

solution or passage of the stones. The rise of the sulfapyridine blood level in advance of that of the nonprotein nitrogen level and the persistence of the high sulfapyridine level is explained by reabsorption of this drug from the tubules.

The incidence of uroliths and also of lesions referable to them was surprisingly small in the present experimental series as compared to previous experiments.[6] This discrepancy can be explained only on the basis of spontaneous solution of the uroliths[8] caused by the interruptions in the drug treatments over the various week-ends.

Conclusions

1. The prolonged administration of sulfapyridine in excessive doses was followed by (but not the cause of) glomerular lesions in partially nephrectomized white rats. The greater the amount of kidney tissue removed surgically prior to administration of the drug, the more frequent and the more severe were the lesions which were found.

2. The glomerular lesions consisted of swelling and proliferation of capsular epithelium, thickening of basement membranes of glomerular capillaries, focal hyaline degeneration of the tufts, endothelial and epithelial proliferation in glomerular tufts and focal adhesions of tufts to Bowman's capsule in foci of capsular epithelial proliferation.

3. Identical lesions were produced in rats by the resection of appropriate amounts of renal parenchyma without resorting to additional procedures.

4. Sulfapyridine does not appear to have been directly concerned in the etiology of the glomerulitis in the drug-treated rats. It appears more likely that the production of the lesions was related to the excessive work-demand placed upon the glomeruli remaining after surgical resection of renal tissue and after tubular obstruction by sulfapyridine uroliths.

5. The glomerular lesions observed resemble those caused by nephrotoxic sera or by a high protein diet.

References

1. Smadel, J. E. Experimental nephritis in rats induced by injection of antikidney serum. III. Pathological studies of the acute and chronic disease. *J. Exper. Med.*, 1937, **65**, 541–555.

2. Horn, Henry. The experimental nephropathies. *Arch. Path.*, 1937, **23**, 71–121.

3. Medlar, E. M., and Blatherwick, N. R. The pathogenesis of dietary nephritis in the rat. *Am. J. Path.*, 1937, **13**, 881–895.

4. Jaffe, R. H. Spontaneous chronic glomerulonephritis in a rat. *Tr. Chicago Path. Soc.*, 1928–31, **13**, 160–162.

5. Gross, Paul; Cooper, F. B., and Scott, R. E. Urolithiasis medicamentosa. *Urol. & Cutan. Rev.*, 1940, **44**, 205–209.

6. Gross, Paul; Cooper, F. B., and Lewis, Marion. Urinary calculi caused by sulfapyridine. *Urol. & Cutan. Rev.*, 1939, **43**, 299–302.

7. Antopol, William, and Robinson, Harry. Pathologic and histologic changes following oral administration of sulfapyridine. With a short note on sodium sulfapyridine. *Arch. Path.*, 1940, **29**, 67–76.

8. Gross, Paul; Cooper, F. B., and Lewis, Marion. The fate of urinary calculi caused by the administration of sulfapyridine. *Urol. & Cutan. Rev.*, 1939, **43**, 439–441.

DESCRIPTION OF PLATE

PLATE 15

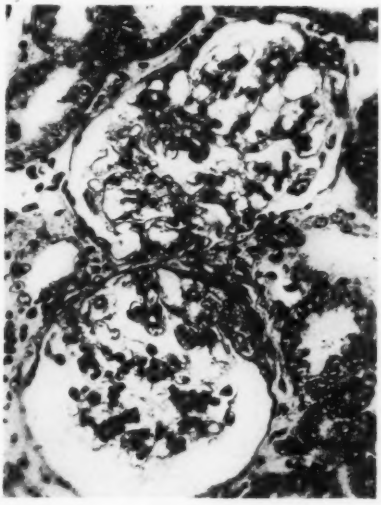
FIG. 1. Glomeruli showing proliferation of capsular epithelium and adhesions of tufts to capsule. From a rat treated for 11 weeks with sulfapyridine following removal of one and one-half kidneys. Hematoxylin and eosin stain. × 620.

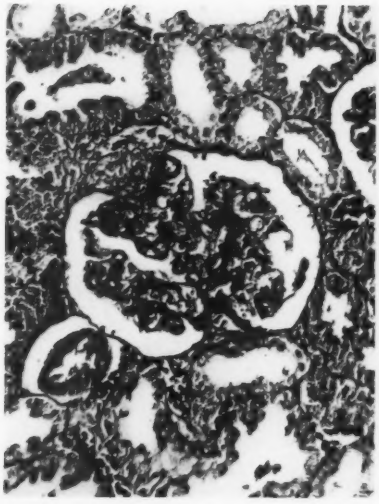
FIG. 2. Glomerulus showing reduplications of the basement membrane in the region of glomerular adhesion. The basement membrane of the tuft is thickened. From the same animal as Figure 1. Azocarmine stain. × 620.

FIG. 3. Glomerulus showing focal proliferation of capsular epithelium, adhesion of tuft and swelling and hyalinization of the cells in the upper portion of the tuft. From a control rat not treated with sulfapyridine but subjected to removal of somewhat more than one and one-half kidneys. Sacrificed 40 days after the last operation. Hematoxylin and eosin stain. × 620.

FIG. 4. Glomerulus showing proliferation and swelling of capsular epithelium, adhesions of the tuft, and "bunching" of hyperchromatic, irregularly shaped, shrunken nuclei in the center of the tuft. From the same animal as Figure 3. Hematoxylin and eosin stain. × 620.

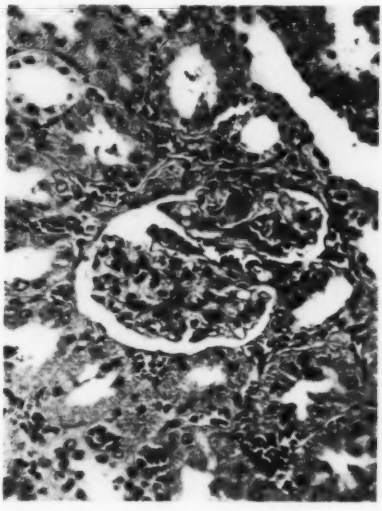
1

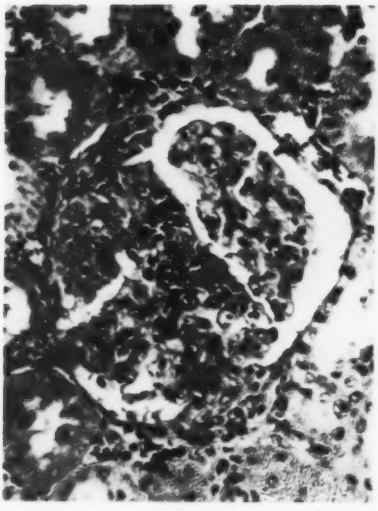




2

3





4

Gross, Cooper and Morningstar Glomerulonephritis and Sulfapyridine

INTERSTITIAL MYOCARDITIS FOLLOWING THE CLINICAL AND EXPERIMENTAL USE OF SULFONAMIDE DRUGS *

A. J. FRENCH, M.D., and C. V. WELLER, M.D.
(From the Department of Pathology, University of Michigan, Ann Arbor, Mich.)

In the routine microscopic examination of autopsy material at the University Hospital (Ann Arbor) during the past 4 years, there were noted occasional examples of interstitial myocarditis, with eosinophilic cellular infiltrations, for which the usual etiologic factors appeared to be lacking. Upon comparing the clinical records of these cases it was found that medication with one or more of the sulfonamide drugs during the terminal illness of each patient was the only factor common to them all. This finding led to two lines of investigation. In one, the autopsy material for the period 1937–1941 was surveyed to determine the incidence of this form of myocarditis in relation to known therapeutic use of the sulfonamide drugs. In the other, daily intraperitoneal introduction of neoprontosil, sulfanilamide, sodium sulfapyridine and sodium sulfathiazole into mice, rats and rabbits, in doses slightly less than those usually employed in clinical medicine, was followed by microscopic examination of the organs of these animals with special attention to the hearts.

LITERATURE

In spite of the voluminous literature relative to the sulfonamide drugs, there is comparatively little histopathologic data available. Observations have been based largely on experimental material and have dealt with the kidneys, liver, spleen and bone marrow. Only a few reports can be found in which the effect of these drugs on the myocardium is considered. Nelson [1] noted myocarditis in a hen which had been given sulfapyridine. The cellular infiltrations were said to be monocytes and polymorphonuclear cells. Eosinophil cells were not mentioned. Dozzi [2] and also Scheinberg and Ingle [3] noted electrocardiographic changes in patients receiving drugs of the sulfonamide group. In each instance, the patient recovered.

Several authors have failed to note changes in the hearts of animals used for toxicologic studies of these drugs. In some of these reports it is not clear to what extent the myocardium was examined histologically. Marshall, Cutting and Emerson [4] reported an absence of histologic changes in dogs, mice and rabbits that had received sulfanilamide. Davis, Harris and Schmeisser [5] gave large doses of sulfanilamide to rats for periods up to 3 months and reported focal necrosis of the liver,

* Received for publication, June 30, 1941.

enlargement of the spleen and sulfanilamide crystals in the exudate in the bronchi. Using rats, Machella and Higgins [6] produced an anemia with sulfanilamide in doses of from 0.25 to 2.0 gm. per Kg. of body weight, given by stomach tube over periods of 10 days to 2 months. The histologic changes were reported as similar to those noted by Davis, Harris and Schmeisser. Molitor and Robinson [7] found no histologic changes with benzylsulfanilamide, but noted splenic enlargement and degenerative changes in the liver and kidneys from sulfanilamide itself. Antopol and Robinson,[8] using sulfapyridine and sodium sulfapyridine in toxic doses, produced urolithiasis in rats, rabbits and monkeys, but not in mice or dogs. Renal histopathology was stressed and no changes were noted in the hearts of the animals examined. Antopol, Lehr, Churg and Sprinz [9] found changes in the livers and kidneys of rats after the intravenous injection of the sodium salts of sulfapyridine, sulfathiazole and sulfamethylthiazole like those previously described by Antopol and Robinson, and others. Stryker [10] found basophilic casts in the renal tubules of animals given sulfapyridine. Toomey, Reichle and Takacs [11] reported similar renal changes in monkeys receiving sulfapyridine in doses comparable to those used in infants. Microscopic lesions were found by Rake, van Dyke and Corwin [12] in the livers and kidneys of mice, rats and monkeys to which sulfathiazole and sulfapyridine were administered with food. Sobin,[13] using large doses of acetylsulfapyridine in rats, produced concretions in renal tubules, as well as dense infiltrations of eosinophils in the submucosal tissues of the renal pelves.

References to the induction of eosinophilic cellular infiltrations and to blood eosinophilia following the use of these drugs have appeared. Hageman,[14] in mice on heavy doses of sulfanilamide, found an increase in the eosinophils of the bone marrow. P'an [15] found eosinophilia of the peripheral blood stream in a dog which had been given large doses of sulfanilamide over a period of several months. However, this dog was found at autopsy to have had tapeworms (*Dipylidium caninum*). Although many organs were studied microscopically, no histologic changes were noted. Kreutzmann and Carr [16] found an increase in the eosinophils of the blood in rabbits on a daily dose of prontosil of 0.25 gm. per Kg. of body weight for 21 days. Interpretation of eosinophilia in rabbits is difficult, but the controls seemed adequate.

Clinical Material

It was ascertained by inspection of the individual case records that 283 of the 1,706 patients upon whom autopsies were performed during the period 1937–1941 had been given one or more of the sulfonamide

drugs during some part of the last few weeks before death. This figure is known to be less than the actual total because a complete record of the medication was not available in every instance. The routine microscopic sections, stained with hemalum and eosin, of the hearts of these 283 patients were reëxamined. In 61 there was no evidence of interstitial myocarditis. These negative findings were not significant in all instances because some of these patients had been given only a few doses of a sulfonamide drug or such medication had so shortly preceded death that it could not have been expected to produce a tissue reaction. Also examination of additional sections from other portions of the myocardium might have reduced the number considered negative. In 40 hearts, interstitial infiltrations were found, but were of so slight a degree that this group was set aside as inconclusive. Infiltrations of this degree were occasionally found without assignable cause before the sulfonamide drugs were introduced. Also excluded from further consideration were 56 hearts from patients in respect to whom there was evidence, either from clinical observation or from the findings at autopsy, of some disease known to result in, or to be associated with, interstitial myocarditis. Thus hearts from cases of bacterial endocarditis, coronary or mural thrombosis, purulent pericarditis, rheumatic heart disease, syphilis, diphtheria, septicopyemia, leukemia, asthma and trichinosis were excluded, although many showed interstitial infiltrations which were rich in eosinophils and were in every respect like those to be described for the group which was retained as significant. After these exclusions there remained a group of 126 cases (44.5 per cent of the original 283) in which a significant interstitial myocarditis was present. For this group, sulfonamide medication was the only factor known to be common to them all. It was therefore believed to be the cause of the myocardial lesion.

When the 56 cases which were excluded from consideration because of the presence of some condition which might produce an interstitial myocarditis are deducted from the original group of 283 cases which had received sulfonamide drugs, the percentage showing an otherwise unexplained myocarditis rises to 55.5.

The patients in this group varied in age from 1 month to 87 years. The acceptable cases were divided about equally as to sex; 70 males and 56 females. The drugs given them included azosulfonamide (prontosil, neoprontosil), sulfanilamide (prontylin), sulfapyridine (dagenan), sulfathiozole and sulfamethylthiazole. The amounts which apparently produced significant infiltrations varied from 5 gm. to more than 200 gm. and were administered over periods of a few hours to several months. No examples of interstitial myocarditis were found in

which the use of the drugs had been discontinued for more than 30 days prior to death. An evaluation of the liability of each particular drug to produce myocardial infiltrations was impossible from the clinical material, for many patients had received several members of the group at different times and for variable periods of time during their terminal illnesses.

As a control series, the hearts from 50 consecutive autopsies performed in 1932 were examined microscopically and all cellular infiltrations noted. This search was conducted without knowledge of either clinical or autopsy records for these cases. The records were subsequently consulted and in this group a factor known to be a causal element in the production of interstitial myocarditis was found to be present in every case in which infiltrations were found. We are fully aware that such a result would not be uniformly obtained because in years past not every case of interstitial myocarditis could be accounted for by one of the conditions used to eliminate cases which might have invalidated the results in the group which received sulfonamide drugs. There have always been cases of "idiopathic" interstitial myocarditis, although this group seems to grow smaller year by year.

The Myocardial Lesion

No macroscopic changes attributable to the use of the sulfonamide drugs were found in the hearts included in this series.

The microscopic features of the lesion in the clinical material included an interstitial myocarditis with the cellular infiltrations chiefly paravascular in distribution but also occurring diffusely in the more marked examples (Figs. 1 and 2). The myocardium was widely involved in both auricles and ventricles. The looser auricular myocardium often exhibited a heavier paravascular infiltration than did the compact ventricular muscle. Some foci were likewise seen in the epicardium, but such cases were included in the positive series only when there was no exudate upon the epicardium and when a characteristic infiltration was found in the myocardium as well.

The cellular infiltrations contained large mononuclear cells of clasmatocytic type and, characteristically, numerous cells with granular eosinophilic cytoplasm. Some of the eosinophils were polymorphonuclear but many appeared to be younger forms of the myeloid series and had a single nucleus and a less granular cytoplasm. Except for the acidophilic cytoplasm, some of them resembled plasma cells.

In occasional hearts the interstitial supporting tissue showed a slight bluish-staining quality with hemalum and eosin. This was considered to be due to mucinous degeneration of connective tissue, but correlation

with either intensity or duration of treatment could not be demonstrated. The significance of this change remained undetermined.

Parenchymatous degeneration in the form of slight Zenker's necrosis was noted but there was no significant simple necrosis and no liquefaction of the muscle. No abscesses were found which could be considered a part of the significant lesion. However, abscess formation was interpreted as evidence of septicemia even though the abscesses were found only in the heart and no positive blood cultures were obtained. Therefore, such cases were automatically excluded from further consideration.

Eosinophilic infiltrations were occasionally noted in *other organs,* particularly in the liver, lungs and kidneys. In some instances the bone marrow, spleen and lymph nodes showed at least a relative increase in eosinophils. The presence of eosinophil cells in these situations may prove to be significant, but no further study of them was made in connection with the immediate problem.

Experimental Production of the Lesion

When the association of interstitial myocarditis, rich in eosinophil cells, with medication with neoprontosil was first noticed in the autopsy material, small groups of mice, rats and rabbits were started upon daily intraperitoneal injections of neoprontosil (the disodium salt of 4-sulfonamido-phenyl-azo-7-acetylamino-1-oxynaphthalene-3:6-disulphonic acid). Dosage was so computed as to be slightly less than the usual human dose of 4 gm. per 24 hours for an adult weighing 70 to 75 Kg. A smaller dose was chosen for experimental introduction in the belief that absorption would be more effective from the peritoneum than from the gastro-intestinal tract. Keeley,[17] however, has found that, although sulfanilamide is absorbed promptly from the peritoneal cavities of dogs, the concentration of the drug in the blood is lower following intraperitoneal injection than other workers have reported as resulting from oral administration. This discrepancy in the expected concentration in the blood has not been explained. It can have no effect upon the significance of the results obtained in the experimental series here reported other than to enhance their value, for the dosage was apparently more conservative than was at first planned and was definitely lower than standard human medication regardless of the mode of introduction.

For the preliminary series, a 5.0 per cent solution of neoprontosil was used for daily intraperitoneal injection in the following amounts: for mice, 0.015 cc.; for rats, 0.15 cc.; for rabbits, 1.5 cc. These animals were sacrificed at intervals and interstitial cellular infiltrations were found in the hearts of many of them.

In view of the positive findings in the preliminary series, larger groups of animals were employed for a second series and sulfanilamide (para-aminobenzene sulfonamide), sodium sulfapyridine (sodium salt of 2-sulfanilamide-6-aminopyridine) and sodium sulfathiazole (sodium salt of 2-sulfanilamide-thiazol) were administered, as well as neoprontosil. The sodium salts of sulfapyridine and sulfathiazole were selected for their greater solubility as compared with the parent products. Neoprontosil, sodium sulfapyridine and sodium sulfathiazole were used in a 5.0 per cent aqueous solution while the less soluble sulfanilamide was in a 0.5 per cent aqueous solution. Daily intraperitoneal injections were made. A small area on the abdominal wall was shaved for an injection site and sterile technic was observed throughout so far as possible. No evidences of either acute or chronic peritonitis were noted at autopsy.

Animals similar to those of the first series were employed. The Swiss mice weighed 20 to 25 gm.; the Wistar stock rats, 200 to 250 gm., and the domestic rabbits of mixed strains, 2,000 to 2,500 gm. All animals used were virginal and males and females were separated to avoid pregnancy as a variable factor. Dosages were computed, as for the first series, to be slightly smaller than the usual human dose. The amount of each drug that was injected at one time is shown in Table I. The

TABLE I

Daily Intraperitoneal Injection Given to Second Experimental Series

	Neoprontosil 5.0% aqueous solution	Sodium sulfapyridine 5.0% aqueous solution	Sodium sulfathiazole 5.0% aqueous solution	Sulfanilamide 0.5% aqueous solution
	cc.	*cc.*	*cc.*	*cc.*
Mice	0.015	0.015	0.015	0.15
Rats	0.15	0.15	0.15	1.5
Rabbits	1.5	1.5	1.5	15.0

mice and rats were killed at intervals of 10, 20 and 30 days after receiving for that period daily intraperitoneal injections of the drug under trial. To additional groups of mice and rats neoprontosil and sulfanilamide were given for 10 days in doses ten times the established sub-normal comparable human dose. Sodium sulfathiazole and sodium sulfapyridine were not available in sufficient quantity for protracted use at this concentration.

As controls, 29 mice, 26 rats and 26 rabbits, all untreated but from the same stock as those treated, and housed and fed in the same manner, were killed and their hearts were examined microscopically. "Spontaneous myocarditis" was found in many of the control rabbits. Miller [18] has reported an incidence of interstitial myocarditis of 60 per cent in control rabbits. Seegal, Seegal and Jost [19] suggested a local

application of the Arthus phenomenon as a possible etiologic factor in the apparently spontaneous interstitial myocarditis of rabbits. These reports, fortified by the findings in our control rabbits, led to the exclusion of the material from rabbits from further consideration. No cellular infiltrations of significance were found in the hearts of either the control mice or rats.

In the second experimental series, an interstitial myocarditis, characterized by numerous eosinophil cells and resembling in all respects that in the clinical material was found in 38 of 60 mice and in 33 of 47 rats. Details as to the duration of administration of the drugs and the distribution of positive findings are shown in Table II. Similar infiltrations were found in 12 of 14 rabbits which were injected at the same time but, as previously stated, these were considered to be of questionable significance.

TABLE II
Second Group

Drug used and duration of experiment		Mice		Rats	
		Total number used	Number with myocarditis	Total number used	Number with myocarditis
Neoprontosil	10 days	4	2	4	3
	20 days	3	4	3	1
	30 days	3	4	3	3
Sulfanilamide	10 days	4	0	4	3
	20 days	3	3	2	2
	30 days	3	3	3	2
Sodium sulfapyridine	10 days	4	4	4	3
	20 days	3	1	2	2
	30 days	3	3	3	3
Sodium sulfathiazole	10 days	4	1	4	3
	20 days	3	3	2	2
	30 days	3	2	3	2
Tenfold dosage					
Neoprontosil	10 days	10	5	10	4
Sulfanilamide	10 days	10	3	0	0
Totals		60	38	47	33

It will be seen that myocardial infiltrations followed the use of each of the drugs administered. In general, the frequency with which myocarditis developed increased with the duration of treatment and with the amount administered. The lesions tended to be more focal in character in the experimental animals than in the human material.

DISCUSSION

As previously stated, no attempt was made to determine from the clinical material whether the various drugs used differed in their effects

upon the myocardium. The use of more than one drug for the same patient and the extreme variability in intensity and in duration of dosage made such an investigation futile. In the experimental series, as they were set up with less than comparable clinical dosage, no significant differences in toxicity were recognized among the four drugs utilized.

No strict correlation between the duration and intensity of medication on the one hand, and the severity of the interstitial myocarditis on the other, could be found in the clinical material. Actually some patients with marked myocardial infiltrations had received relatively small doses of the drugs over short terminal periods in their illnesses. The question of idiosyncrasy to the drugs is thereby raised—a question which seems all the more appropriate in view of the eosinophilic character of the infiltrations. No solution to this question is evident from this work, but a definite avenue of approach is suggested.

That the lesions attributable to sulfonamide drug therapy are not of a character which would be expected to result in irreversible changes is significant and encouraging. Nevertheless, it is impossible to evaluate the effect which such lesions might have on the heart of an individual who was otherwise seriously ill. It is therefore highly desirable that such patients be examined at frequent intervals with reference to their cardiovascular functional efficiency. Electrocardiographic studies might properly parallel sulfonamide drug therapy in many cases.

It is not the intent of this report to discourage the use of the sulfonamide drugs, but to call attention to one of the several by-effects which make it desirable to keep patients, to whom these drugs are being given, under the closest clinical scrutiny and to limit the amount and the duration of administration to the lowest levels consistent with beneficial results.

Summary

An interstitial myocarditis, rich in eosinophil cells, has been found in the hearts of 126 patients whose sole common factor was that one or more of the sulfonamide drugs had been administered shortly before death.

A similar eosinophilic interstitial myocarditis has been produced in mice and rats by the daily intraperitoneal injection of neoprontosil, sulfanilamide, sodium sulfapyridine and sodium sulfathiazole in amounts computed to be less than comparable to the usual human dosage.

Eosinophilic infiltrations were seen also in the lungs, livers and kidneys of both the human cases and of the experimental animals. The

bone marrow, spleen and lymph nodes likewise showed increased eosinophil cells in some instances.

These observations suggest the advisability of greater precaution in the use of the sulfonamide drugs.

NOTE: A preliminary report upon this investigation appeared in *Proc. Soc. Exper. Biol & Med.*, 1941, **48**, 1–3. Differences in numerical results in the experimental sections of the two reports are due to combining series in the preliminary report and to slight changes in diagnostic criteria considered to be significant.

REFERENCES

1. Nelson, A. A. Histopathological changes in hens and rabbits following administration of sulfanilamide and sulfanilyl sulfanilamide (di-sulfanilamide). *Pub. Health Rep.*, 1939, **54**, 106–127.
2. Dozzi, D. L. Transient nodal rhythm following use of sulfanilamide. *Am. J. M. Sc.*, 1938, **195**, 771–773.
3. Scheinberg, D., and Ingle, C. W. Possible myocardosis due to sulfanilamide. *Memphis M. J.*, 1939, **14**, 87–88.
4. Marshall, E. K.; Cutting, W. C., and Emerson, Kendall. The toxicity of sulfanilamide. *J. A. M. A.*, 1938, **110**, 252–257.
5. Davis, H. A.; Harris, L. C., and Schmeisser, H. C. Toxic effects of sulfanilamide upon tissues of rats. *J. Lab. & Clin. Med.*, 1940, **25**, 1263–1275.
6. Machella, T. E., and Higgins, G. M. Anemia induced in rats by means of sulfanilamide. *Am. J. M. Sc.*, 1939, **198**, 804–815.
7. Molitor, H., and Robinson, H. Some pharmacological and toxicological properties of sulfanilamide and benzylsulfanilamide. *J. Pharmacol. & Exper. Therap.*, 1939, **65**, 405–423.
8. Antopol, William, and Robinson, Harry. Urolithiasis and renal pathology after oral administration of 2 (sulfanilylamino) pyridine (sulfapyridine). *Proc. Soc. Exper. Biol & Med.*, 1939, **40**, 428–430. *Ibid.* Pathologic and histologic changes following oral administration of sulfapyridine. With a short note on sodium sulfapyridine. *Arch. Path.*, 1940, **29**, 67–76.
9. Antopol, William; Lehr, David; Churg, Jacob, and Sprinz, Helmuth. Changes in the urinary tract and other organs after administration of three sulfanilamide derivatives. *Arch. Path.*, 1941, **31**, 592–602.
10. Stryker, W. A. The nature of the renal lesion with sulfapyridine therapy. *J. A. M. A.*, 1940, **114**, 953–954.
11. Toomey, J. A.; Reichle, H. S., and Takacs, W. S. Effects upon monkeys of sulfapyridine in doses comparable with those used for infants. *J. Pediat.*, 1940, **16**, 179–190.
12. Rake, Geoffrey; van Dyke, H. B., and Corwin, W. C. Pathologic changes following prolonged administration of sulfathiazole and sulfapyridine. *Am. J. M. Sc.*, 1940, **200**, 353–362.
13. Sobin, S. S. Personal communication.
14. Hageman, P. O. Toxicity of sulfanilamide. A study of the pathological lesions in white mice. *Proc. Soc. Exper. Biol. & Med.*, 1937–38, **37**, 119–122.

15. P'an, S. Y. Observations on the chronic effect of sulfanilamide in dogs and monkeys with particular reference to the blood. *Chinese M. J.*, 1939, **56**, 111–121.

16. Kreutzmann, W. B., and Carr, J. L. Effect of prontosil on blood cells. *Proc. Soc. Exper. Biol. & Med.*, 1938, **38**, 19–21.

17. Keeley, J. L. Intraperitoneal administration of sulfanilamide; concentration in peripheral blood in dogs: *Proc. Soc. Exper. Biol. & Med.*, 1941, **46**, 458–462.

18. Miller, C. P., Jr. Spontaneous interstitial myocarditis in rabbits. *J. Exper. Med.*, 1924, **40**, 543–551.

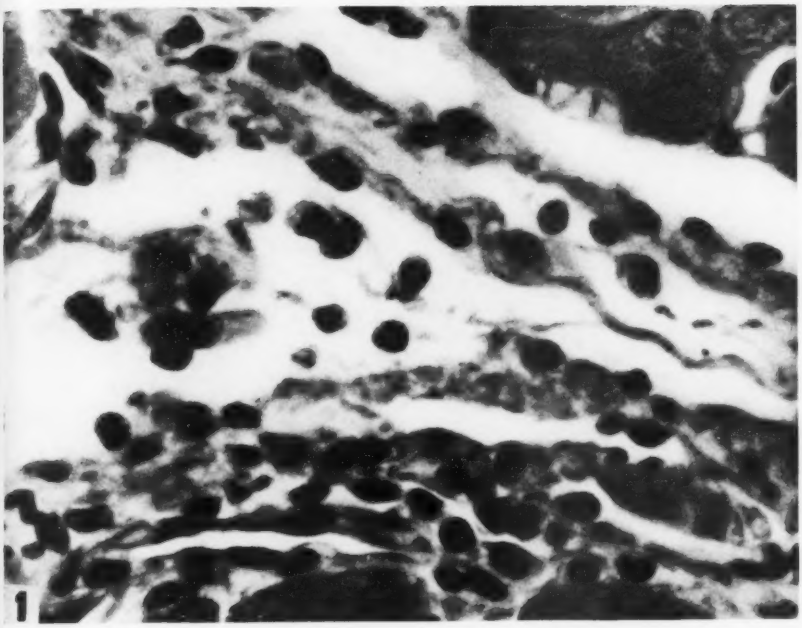
19. Seegal, D.; Seegal, B. C., and Jost, E. L. The Arthus phenomenon. Local anaphylactic inflammation in rabbit pericardium, heart, and aorta. *J. Exper. Med.*, 1932, **55**, 155–162.

DESCRIPTION OF PLATES

PLATE 16

FIG. 1. Interstitial myocarditis as found in the heart of a woman 48 years old (A-91-AS) who had received 4 gm. of neoprontosil daily for the terminal 4 days of life. This was given in an effort to control a postoperative infection. Hemalum and eosin stain. × 410.

FIG. 2. Interstitial myocarditis as found in the heart of a man of 72 years. For infection associated with prostatic obstruction, he had been given a total of 50 gm. of sulfanilamide during the last 2 weeks of life. Hemalum and eosin stain. × 400.


1

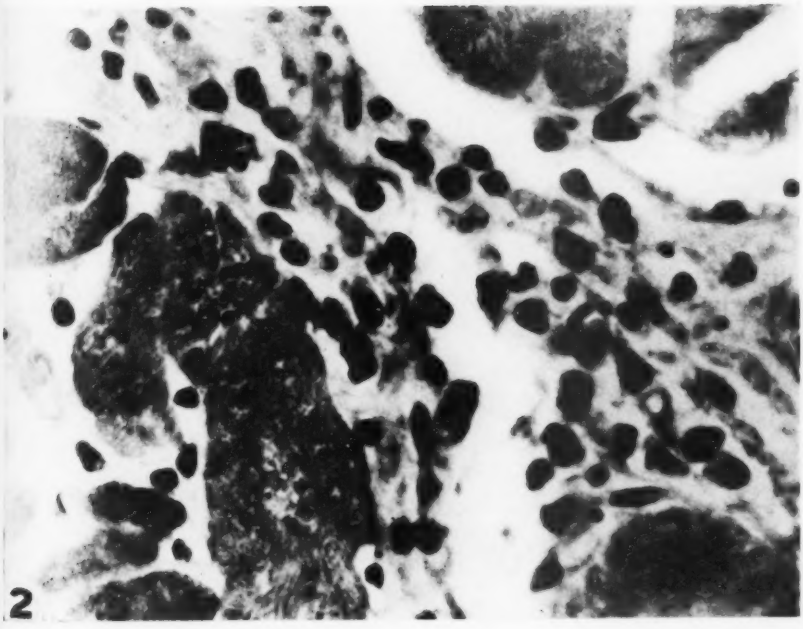
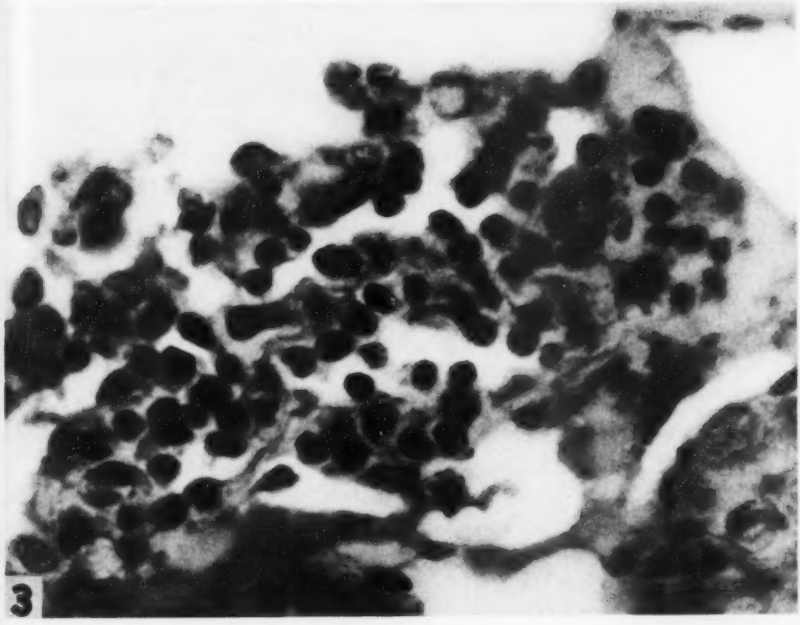
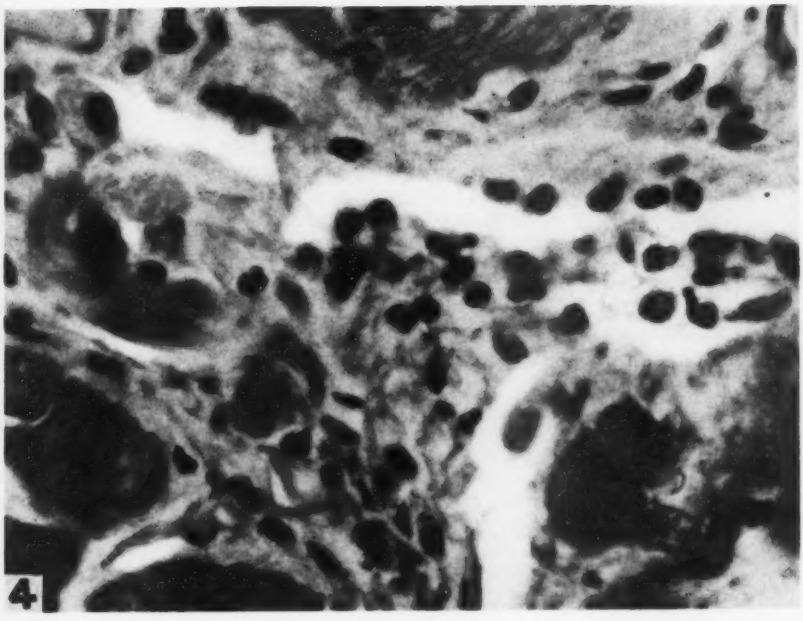

2

PLATE 17

FIG. 3. Subendocardial infiltration photographed from the heart of rat no. 61, which had received daily intraperitoneal injections of sulfanilamide for 10 days. Hemalum and eosin stain. × 390.

FIG. 4. Interstitial myocardial infiltration in the heart of mouse no. 72, which had received daily intraperitoneal injections of neoprontosil for 20 days. Hemalum and eosin stain. × 390.





French and Weller Myocarditis Following Sulfonamide Drugs

BRONCHIOGENIC CARCINOMA IN ASSOCIATION WITH PULMONARY ASBESTOSIS *

Report of Two Cases

Herbert B. Holleb, M.D., and Alfred Angrist, M.D.
(From the Department of Pathology, Queens General Hospital, Jamaica, N. Y.)

Following the report of a case of carcinoma of the lung in the presence of pulmonary asbestosis by Lynch and Smith [1] in 1935, several similar papers appeared in rapid succession. In the same year, Gloyne[2] presented two cases of squamous-celled carcinoma of the lung occurring in asbestosis, and in the following year,[3] a case of oat-celled carcinoma. Egbert and Geiger [4] reported one case in 1936. Two years later, Nordmann [5] analyzed the recorded cases after adding two of his own to the literature. His views on the relationship between the two diseases were revealed by the title of his paper, "Der Berufkrebs der Asbestarbeiter" [Occupational Cancer of the Asbestos Worker]. The eighth case was reported by Lynch and Smith [6] together with an additional case of squamous metaplasia of the bronchial epithelium in an asbestotic lung. The two cases here reported bring the total number recorded in the literature to ten.

Reports of Cases

Case 1

W. M., a white male, 52 years old, was admitted to Queens General Hospital complaining of a chronic morning cough which for many years had been productive of small amounts of tenacious sputum. During the 4 weeks prior to admission, the cough became progressively more severe and he was compelled to stop work. The sputum now amounted to approximately 4 oz. (120 cc.) each day and was occasionally blood-streaked. This was accompanied by right chest pain, marked anorexia and a weight loss of about 15 lbs. (6.5 Kg.).

There was no history of past medical, surgical or venereal disease except for a nasal operation at the age of 25.

For the past 25 years the patient had been employed almost continuously by various manufacturers of asbestos products. His duties always entailed the covering of steam and water pipes with asbestos fabric. He stated that many of his co-workers had died of "silicosis."

Physical examination revealed a weakened, emaciated, white male breathing noisily and with difficulty, and coughing frequently. The neck veins were distended. There were signs of consolidation over the upper right portion of the chest anteriorly. The breath sounds were distant over the lower posterior areas on both sides of the chest. The cardiac apex and the trachea appeared shifted to the right. There were no other unusual findings in the heart, abdomen, extremities or nervous system.

Laboratory Data. Temperature, 99.8° F. Pulse, 124 per min. Respirations, 22 per min. Blood pressure, 110/80. Urine, negative except for occasional red

* Received for publication, May 19, 1941.

and white blood cells. Blood count, 4,800,000 red cells per cmm. and 11,000 white cells per cmm., of which 72 per cent were polymorphonuclear cells. Hemoglobin, 12.3 gm. per 100 cc. Venous pressure, 140 mm. of blood. Ether circulation time, 15 sec. Saccharin circulation time, 3 min. (probably unreliable). The sputum was negative for acid-fast bacilli. A Kline test of the blood was negative.

Roentgenograms of the chest taken shortly after admission showed a large area of diminished illumination involving the entire right upper lobe with "patchy infiltration of the lower lobe." The lower cardiac shadow was deviated to the right. The left lung field presented prominent pulmonary markings which, however, could not be regarded as definitely pathological. On the fifth hospital day bronchoscopy revealed a sessile mass protruding from the posterior wall of the trachea about 1.5 cm. above the carina, leaving a narrow crescentic lumen about 2 mm. in width. Biopsy from this region showed bronchial tissue with only an inflammatory reaction. During the next 12 days the patient received small daily doses of high-voltage roentgen-ray therapy to the anterior aspect of the chest, totaling 2,600 r. This was accompanied by a marked improvement in respiration. The intratracheal mass had now been reduced in size sufficiently to allow passage of the bronchoscope into the right main bronchus, where ulceration and constriction were noted. Biopsy from this region showed definite carcinoma.

During the first 4 weeks in the hospital he had a low-grade fever which gradually declined. On the 30th hospital day, his temperature rose to 105.2° F. and he complained of a sore throat. Blood cultures taken at this time and repeated 5 days later showed the presence of hemolytic streptococci. In spite of sulfapyridine and, later, sulfanilamide therapy, his temperature remained elevated (101° to 104° F.). He grew rapidly weaker and expired on the 36th hospital day.

The final clinical diagnoses were bronchiogenic carcinoma in the region of the carina, *Streptococcus hemolyticus* septicemia and terminal pulmonary edema.

Postmortem Findings. The pertinent descriptive data are limited essentially to the thorax.

The right pleural cavity was completely obliterated by dense fibrous adhesions. The left pleural cavity contained approximately 200 cc. of watery yellow fluid with numerous flakes of fibrin. A shaggy yellow coating covered the posterior parietal pleura. Section of the right lung (Fig. 1) revealed an irregular, globular, hard, grayish white mass 5 cm. in diameter. The mass had completely eroded and encircled the right main bronchus. Some portions of the mass were softened and had been partially excavated. Grayish white neoplastic tissue was seen infiltrating beneath intact bronchial epithelium upward to a point 2.5 cm. above the carina, and downward along the right main bronchus for a distance of 5 cm. beyond the lower margin of ulceration. The remainder of the right upper lobe presented several prominent, irregular, grayish white septa extending out to the pleura which measured 3 mm. in thickness in this region, and was composed of firm, opaque, white tissue. The parenchyma of the right upper and middle lobes presented numerous pneumonitic areas. The cut surfaces of the right lower lobe and the entire left lung were grayish red and crepitant.

Within the mediastinum, at the level of the bifurcation of the

trachea, there was an irregular, nodular mass of necrotic neoplastic tissue 8 cm. in diameter which was adherent to and compressed both trachea and esophagus. (This probably corresponded to the mass visualized bronchoscopically.) The pulmonary vessels were intact except for the main branch to the right upper lobe which was so compressed by the intrapulmonary tumor mass that a probe was passed only with difficulty.

Within the pericardial sac there was approximately 300 cc. of fluid similar to that found in the left pleural cavity. A thick, shaggy, yellowish gray coating covered both visceral and parietal pericardium. The myocardium of the right ventricle was 6 mm. thick.

The only extrathoracic metastasis seen grossly was a mass measuring 4 by 2 cm. at the lower pole of the right kidney. Multiple sections of the brain revealed no metastasis. The right adrenal gland showed a cortical adenoma measuring 3 cm. in diameter.

Microscopical study showed that the neoplastic tissue in the lung was composed chiefly of fairly large polyhedral cells with moderately small, round hyperchromatic nuclei. There was no evidence of either intercellular bridges or of "pearl" formation. Many areas showed considerable variation in the size and shape of these cells and in some, large multinucleated cells were present. In a few regions these cells were spindle-shaped and were arranged in cordlike bundles. There was a definite tendency to invade along the alveolar walls, which occasionally gave a superficial resemblance to large glands. Lymphatic permeation was prominent throughout all involved areas. Small islands of neoplastic tissue were present beneath intact bronchial epithelium, corresponding to the picture seen grossly. Many of these islands were in the lumina of distended lymphatics of the lamina propria (Fig. 2).

Small and large masses of the neoplasm, many of which were necrotic, were found within the wall of the bronchus to the right upper lobe, the adjacent lung tissue, the mediastinal lymph nodes and the right kidney. A routine section revealed a minute metastasis lying in the substance of the adrenal cortical adenoma.

Of interest was the presence of squamous metaplasia of the epithelium of the right main bronchus and of several of the bronchial mucous-gland ducts in this region. Distinct intercellular bridges were seen. Structure was slightly atypical, but there was no definite malignant change in the metaplastic epithelium.

Many sections of both lungs showed considerable diffuse interstitial fibrosis, numerous foamy mononuclear cells within the alveolar spaces and a moderate number of golden yellow asbestosis bodies (Fig. 3). These varied from rod-shaped "early" forms to beaded "weathered"

forms. Many were partially or completely engulfed by mononuclear and multinuclear macrophages. Sections treated with potassium ferrocyanide gave positive results for iron. In several regions the alveoli were filled with polymorphonuclear cells and fibrin. In areas of fibrosis a marked obliterative endarteritis was present.

Chemical examination yielded 240 mg. of silicon dioxide per 100 gm. of dried lung.

The final diagnoses were: Bronchiogenic, non-keratinizing, squamous-celled carcinoma of right upper lobe; metastases to mediastinal lymph nodes, to right kidney and to a cortical adenoma of right adrenal gland; pulmonary asbestosis; right ventricular hypertrophy; squamous metaplasia of bronchus; bronchopneumonia of right upper and middle lobes; left pleural effusion; fibrinous pericarditis (*Streptococcus hemolyticus*) with effusion.

Case 2

The history was obtained from the patient's son after the autopsy was completed. His knowledge of some details was admittedly inaccurate.

E. R., a white male, 58 years old, began to experience dyspnea on exertion approximately 15 years before his death. This progressed slowly but steadily so that during his last few years of life he was almost completely exhausted after climbing only one flight of stairs. He had frequent bouts of coughing which were especially severe in the morning but occurred throughout the day and night. This was productive of moderately large amounts of sticky brown sputum which was never blood-streaked. During the spells of coughing, "wheezing" noises in his chest could be heard many feet away. After several years, he was examined by a physician who made a roentgenogram of his chest and told him he had "asbestosis of the lungs." There was no history of cyanosis, orthopnea, paroxysmal dyspnea, angina pectoris or ankle edema.

Several months before his death the patient began to lose weight rapidly. He complained of continual fatigue and his cough became more frequent and more severe. There was no change in the character of his sputum. A physician told the family that the patient had developed "complications following asbestosis." He sank slowly, became comatose and finally expired.

The patient had been employed by a manufacturer of asbestos products since the age of 23 years until a few months before his death. During the first 25 years of this period he was employed as a pipe-coverer, the same occupation as in case 1. Although there was no known association between the two men, the conditions under which they worked were essentially the same. The patient's work called for covering steam and water pipes with asbestos. He was seldom in the same place for more than a few days or weeks, but the work usually was done in unventilated cellars. Large amounts of powdered asbestos cement were used in addition to the asbestos fabric. The patient's son worked with his father for a year but quit because the air was "too dusty" and because several of the employees were "getting sick." During the 10 years before his death, the patient was employed as a salesman and was not exposed to asbestos dust.

Postmortem Findings. Both pleural cavities were almost completely obliterated by fibrous adhesions between the pleural surfaces. The cut surface of the right lung presented a diffuse infiltration of firm white neoplastic tissue replacing most of the lower lobe. There was prominent encirclement of the bronchi and invasion of the septa by the new growth (Fig. 4). Posteriorly, the adjacent tissue of the right upper lobe showed similar parenchymal, peribronchial and septal infiltration by neoplasm. At the apex of the right upper lobe there was an area of fibrous induration and distortion. No actual specific primary bronchial site could be established, but the major location of the tumor-tissue suggested origin in the right lower lobe.

The remaining lobes of both lungs presented numerous, small, firm, irregular greenish black nodules.

Numerous metastatic foci were present beneath the pleura of the right lung, in the mediastinal and peripancreatic lymph nodes, cerebral cortex, liver, kidneys, thoracolumbar vertebrae and several ribs.

No other noteworthy lesions were present in the remaining viscera.

Microscopical Study. The neoplasm was composed of moderately large, round to oval cells with round, dense, hyperchromatic nuclei. There was considerable variation in the size and shape of the cells with varying amounts of cytoplasm. Many cells contained several nuclei. There was a compact grouping of the neoplastic cells between fine fibrous trabeculae producing a somewhat alveolar arrangement. No true glandular or squamous features were noted.

In the sections of bronchi studied, no ulceration of the mucosa was present. In many regions, however, there was infiltration of tumor-tissue into the lamina propria with invasion and destruction of the remainder of the bronchial wall (Fig. 5). Many of the large neoplastic masses were necrotic. Lymphatic permeation was prominent in all sections. The metastases presented similar histological and cytological characteristics throughout, but the multinuclear tendency was more marked in the liver. The carcinoma cells in the cerebral cortex were less compactly arranged and showed extensive necrosis and hemorrhage.

Sections of lung showed a widespread interstitial fibrosis somewhat unevenly distributed. Only occasional asbestosis bodies were found (Fig. 6). They were smaller but similar to those seen in case 1. Only a few were seen within large macrophages. Sections treated with potassium ferrocyanide were positive for iron.

Additional findings in the lungs included marked hypertrophy of the muscular coat of the small and large bronchi, obliterative pulmonary endarteritis with recanalization, pulmonary edema and organizing fibrinous exudate on the pleural surfaces.

TABLE I

Summary of Reported Cases of Pulmonary Asbestosis and Carcinoma

Authors	Year	Sex and age	Occupation	Duration of exposure	Freedom from exposure before death	Nature of tumor	Primary site	Metastases
*1. Lynch and Smith	1935	M 57	Weaver	21 yrs.	4 mos.	Squamous-celled	Right lower lobe	Many nodules in right lower lobe
*2. Gloyne	1935	F 35	Spinner	8 yrs.	9 yrs.	Squamous-celled	Right upper lobe	Pleura
*3. Gloyne	1935	F 71	Mattress and opening depts.	19 mos.	15 yrs.	Squamous-celled	Right lower lobe	None
4. Egbert and Geiger	1936	M 41	Weaver	17 yrs.	2 yrs.	Glandular	Left lower lobe	Widespread
*5. Gloyne	1936	M 59	Packer, stores department	10½ yrs.	? mos.	Oat-celled	Left lower lobe	Left upper lobe and pleura
*6. Nordmann	1938	F 35	Carder, spinner, weaver	7 yrs.	9 yrs.	Squamous-celled	Left lower lobe	Liver, kidneys
*7. Nordmann	1938	M 55	Pre-spinning assembly room	7 yrs.	12 yrs.	Squamous-celled	Left lower lobe	Widespread
8. Lynch and Smith	1939	M 50	Weaver	13 yrs.	3 yrs.	Squamous-celled with glandular features	Right lower lobe	Pleura, mediastinal nodes
9. Holleb and Angrist	1941	M 52	Pipe insulator	25 yrs.	9 wks.	Non-keratinizing squamous-celled	Right upper lobe	Mediastinal nodes, kidney, adrenal
10. Holleb and Angrist	1941	M 58	Pipe insulator	25 yrs.	10 yrs.	Oat-celled	Right lower lobe	Widespread, including brain

*Included in previous tabulation by Nordmann.

Chemical examination yielded 4.5 mg. of silicon dioxide per 100 gm. of dried lung.

The final diagnoses were: Bronchiogenic, large oat-celled carcinoma of the right lower lobe with extension to right upper lobe and metastases to mediastinal and peripancreatic lymph nodes, liver, kidneys, cerebral cortex, ribs and thoracolumbar vertebrae; pulmonary asbestosis; chronic bronchitis; tuberculous apical scar of right upper lobe.

Comment

No reference could be found in the literature providing strict criteria for the diagnosis of pulmonary asbestosis. Silica has been shown [7] to constitute a significant, though somewhat variable, portion of the asbestos fiber (39 to 51.5 per cent). Determinations of silica were therefore carried out with the idea that the demonstration of definitely increased values would furnish final confirmation of the diagnosis of asbestosis. This was considered justified since there was no reason, on the basis of clinical record or autopsy findings, to suspect appreciable exposure to any of the silicates other than asbestos.

The dried lungs of case 1 were found to contain 240 mg. per cent of silica, while those of case 2 yielded only 4.5 mg. per cent. McNally [8] and Kettle and Archer [9] stated that lungs may contain as much as 200 mg. per cent without showing histological evidence of silicosis. If this value were used as a standard, the silica content in case 1 would be only slightly elevated while case 2 definitely would fall within known nonpathological limits. The diagnosis of pulmonary asbestosis in both cases must, therefore, rest on the usual criteria: (1) A history of prolonged exposure to the dust—25 years in both cases; (2) the presence of chronic respiratory symptoms, productive cough in one and cough and severe exertional dyspnea in the other without cardiac disease; (3) histological findings of asbestosis; namely, diffuse, interstitial, pulmonary fibrosis, asbestosis bodies, macrophages and giant cells.

The apparent discrepancy between the presence of pulmonary asbestosis and the absence of elevated silica values can be resolved. First, it is known that inhaled asbestos fibers undergo dissolution and phagocytosis, and may eventually disappear entirely, leaving behind irreparable damage.[10] An additional, though probably minor factor, is the elimination of asbestos by expectoration. This occurs not only as fibers but in the form of asbestosis bodies, showing that a tissue reaction has already occurred in the lung.[11] Second, both of these individuals were employed for unusually long periods in occupations in which the exposure to asbestos dust was relatively low. It is therefore conceivable that extensive damage could result as a summation effect,

without appreciable accumulation of asbestos in the lung at any one time. The combination of both factors may account for the particularly low silica content in case 2 in which there was, in addition, freedom from exposure for 10 years before death.

Nordmann [5] presented in tabular form the relevant data on his own two cases of pulmonary asbestosis and carcinoma, and of the four cases previously recorded. After analyzing the results, he came to the conclusion that pulmonary cancer is an occupational disease among asbestos workers. Gloyne,[12] in a recent text, stated that no definite conclusions can be drawn on the relation between these two diseases until more material is available. Table I presents a tabulation similar to Nordmann's, but brought up to date by the addition of four cases, including the two here reported. It should be made clear that the data are presented merely as a summary and not necessarily as supportive evidence for Nordmann's conclusions.

It may be of interest to review briefly, for comparison, the incidence of carcinoma in the material examined in this department. The figures are derived from the autopsy material of a general city hospital for acute illnesses, with 696 beds of which 498 are devoted to adults. In a total of 2,451 routine autopsies performed in a 5-year period, there were 401 instances of carcinoma of all types. This is an incidence of 16.36 per cent. Of these, 59 were bronchiogenic, comprising 14.71 per cent of all carcinomas and 2.41 per cent of the routine autopsies. It was striking, although naturally of no statistical significance, to find bronchiogenic carcinoma in the only two cases of pulmonary asbestosis seen here.

Conclusions

1. Two cases of bronchiogenic carcinoma in association with pulmonary asbestosis are presented.

2. An attempt to demonstrate unusual amounts of silica in the lungs of both cases was unsuccessful.

3. An explanation for the absence of elevated silica values in the presence of asbestosis is offered.

4. The relevant data of all reported cases of pulmonary asbestosis and carcinoma are presented in tabular form.

Note: The authors wish to express their grateful appreciation to Goodwin A. Distler for the clinical data and to Richard Grimes and Howard W. Neail, of the Office of the Chief Medical Examiner, for the opportunity to study the autopsy material.

REFERENCES

1. Lynch, K. M., and Smith, W. A. Pulmonary asbestosis. III: Carcinoma of lung in asbesto-silicosis. *Am. J. Cancer,* 1935, **24,** 56–64.
2. Gloyne, S. R. Two cases of squamous carcinoma of the lung occurring in asbestosis. *Tubercle,* 1935–36, **17,** 5–10.
3. Gloyne, S. R. A case of oat cell carcinoma of the lung occurring in asbestosis. *Tubercle,* 1936–37, **18,** 100–101.
4. Egbert, D. S., and Geiger, A. J. Pulmonary asbestosis and carcinoma. Report of a case with necropsy findings. *Am. Rev. Tuberc.,* 1936, **34,** 143–150.
5. Nordmann, Martin. Der Berufskrebs der Asbestarbeiter. *Ztschr. f. Krebsforsch.,* 1938, **47,** 288–302.
6. Lynch, K. M., and Smith, W. A. Pulmonary asbestosis. V. A report of bronchial carcinoma and epithelial metaplasia. *Am. J. Cancer,* 1939, **36,** 567–573.
7. Sundius, N., and Bygden, A. Der Staubinhalt einer Asbestosislunge und die Beschaffenheit der sogenannten Asbestosiskörperchen. *Arch. f. Gewerbepath. u. Gewerbehyg.,* 1937, **8,** 26–70.
8. McNally, W. D. Silicon dioxide content of lungs in health and disease. *J. A. M. A.,* 1933, **101,** 584–587.
9. Kettle, E. H., and Archer, H. E. The differential diagnosis of tuberculosilicosis. *Proc. Roy. Soc. Med.,* 1933, **26,** 811–824.
10. Lynch, K. M. Pulmonary asbestosis. IV. The asbestos body and similar objects in the lung. *J. A. M. A.,* 1937, **109,** 1974–1978.
11. Gloyne, S. R. In: Lanza, A. J. Silicosis and Asbestosis. Oxford University Press, London, 1938, p. 238.
12. *Idem,* p. 245.

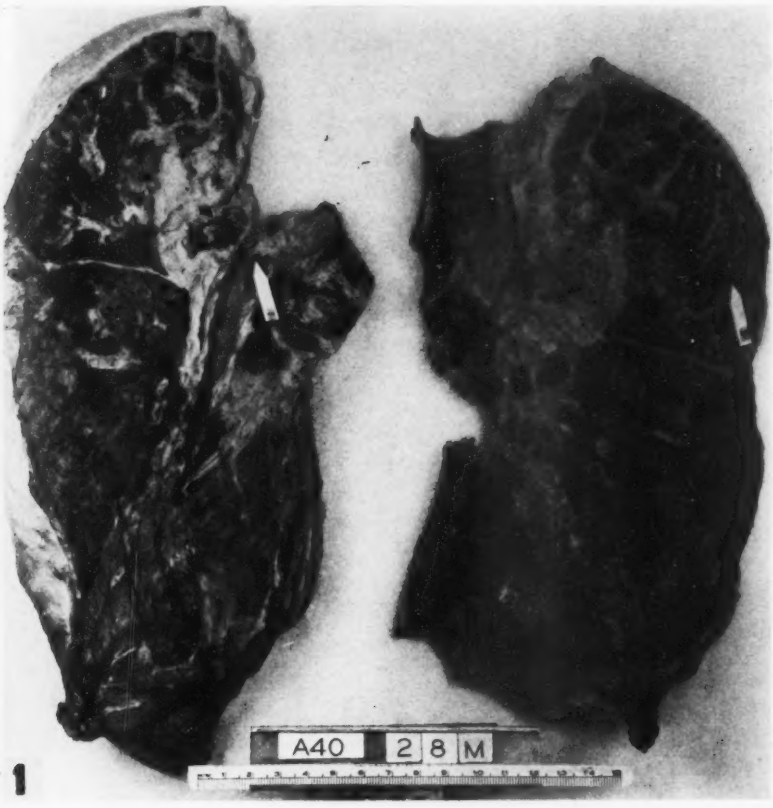
DESCRIPTION OF PLATES

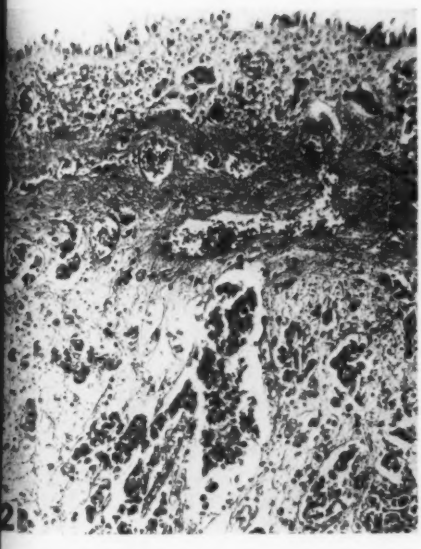
PLATE 18

FIG. 1. Case 1. Hemisected right lung showing ulceration of main bronchus, partially excavated tumor mass in upper lobe, submucosal extension along bronchus, and invasion along interlobular septa.

FIG. 2. Case 1. Right main bronchus showing infiltration of tumor beneath mucosa. × 117.

FIG. 3. Case 1. Large asbestosis body with partial encirclement by giant cell. × 525.


A40
2 8 M
1


2

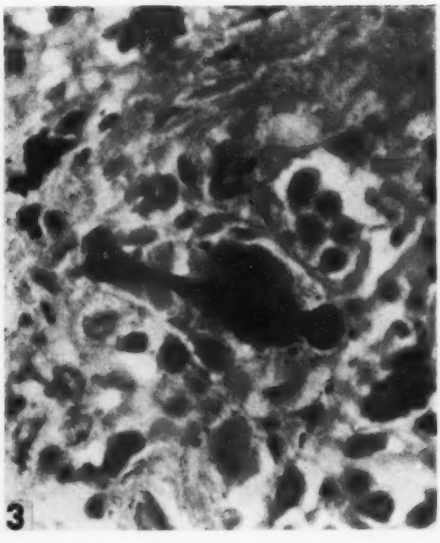
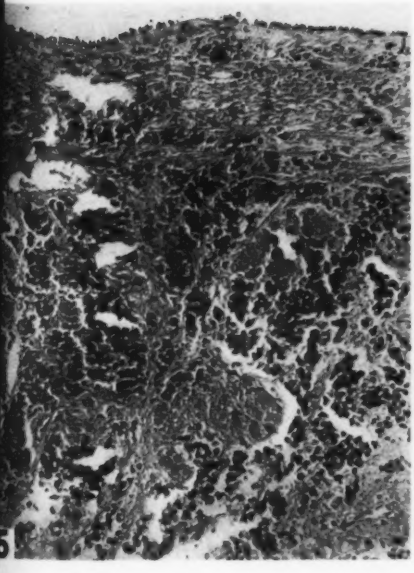

3

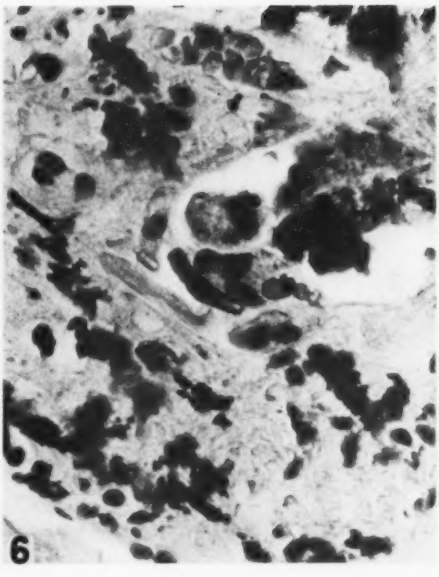
PLATE 19

FIG. 4. Case 2. Right lung showing diffuse carcinoma involving lower lobe and adjacent portion of upper lobe, with prominent encirclement of bronchi by neoplastic tissue.

FIG. 5. Case 2. Bronchus of right lower lobe showing intact basement membrane with attached epithelial layer and underlying diffuse carcinomatous invasion. × 122.

FIG. 6. Case 2. Lung showing several asbestosis bodies. × 547.





Holleb and Angrist

Bronchiogenic Carcinoma and Pulmonary Asbestosis

SECONDARY CARCINOMA IN CIRRHOSIS OF THE LIVER *

JAMES R. LISA, M.D., CYRIL SOLOMON, M.D., and EVERETT J. GORDON, M.D.
(From the Pathological Laboratory, City Hospital, Welfare Island, Department of Hospitals, New York, N. Y.)

This communication reports six cases of secondary carcinoma occurring in livers affected by diffuse cirrhosis. In each instance, the extent of the cirrhosis excluded the possibility that it was associated with the malignant disease and supported the interpretation that it preceded the presence of secondary nodules.

A careful search of medical indexes revealed only five authentic cases of malignant disease metastasizing to cirrhotic livers. The first case was observed by Hanot and Gilbert.[1] Their first impression was that the nodules in the liver were metastatic, but because such a lesion was so rare in their experience they reported the case as one of primary carcinoma arising in Laënnec's cirrhosis. Poulain [2] restudied the case and came to the conclusion that the hepatic tumor was metastatic. The patient was a woman, 68 years old, who had gastro-intestinal complaints without vomiting or hematemesis for 1 year. A midline mass was present in the upper abdomen. Necropsy revealed carcinoma of the stomach, lymphatic metastases and Laënnec's cirrhosis with malignant nodules. The gastric and hepatic tumors were identical, columnar-celled carcinomas. Poulain was the first observer to differentiate clearly between primary and secondary malignancy in cirrhosis of the liver. White [3] reported the second case. The patient was a man, 63 years old, with sarcoma of bones, on whom he performed an autopsy in 1885. The case of Achard and Laubry [4] was that of a man, 65 years old, a chronic alcoholic, who gave a history of ascites and digestive disturbances and who later developed alternating diarrhea and constipation. Carcinoma of the ascending colon, metastases to lymph nodes, mesentery and omentum, and Laënnec's cirrhosis with tumor nodules were disclosed by autopsy. The original growth and the hepatic nodules were diagnosed as cylindrical-celled carcinoma. The hepatic metastases were within the fibrous tissue in which bile ducts were discernible and distinct from the malignant growth. Two cases were reported by Colwell.[5] The first was that of a man, 69 years old, with malignant disease of the orbit who had a solitary metastasis. In the second case, that of a woman, 58 years old, who had mammary carcinoma, two metastases were present. The cirrhosis in both instances was of the Laënnec type.

* Received for publication, March 31, 1941.

Reports of Cases

Case 1

The clinical data are not available. Necropsy (no. 434) revealed intense jaundice, Laënnec's cirrhosis, carcinoma of the papilla of Vater, metastases to the liver and acute cholangitis. The metastases were largely in the connective tissue.

Case 2

The patient was a white woman, 55 years old, with carcinoma of the stomach who died from hematemesis. Necropsy (no. 3635) revealed carcinoma of the stomach with metastases to liver, regional lymphatics, adrenals, duodenum and transverse colon. The liver weighed 1850 gm. and showed biliary cirrhosis and metastases replacing hepatic parenchyma.

Case 3

The patient was a white woman, 55 years old, who gave a history of obstructive jaundice with operation for gallbladder disease 2½ years before coming under observation at City Hospital. She died of peritonitis following an exploratory laparotomy at which carcinoma of the head of the pancreas was discovered. At necropsy (no. 4262) the liver showed diffuse biliary cirrhosis and extensive small metastases in the fibrous tissue extending into the hepatic nodules. Proliferating bile ducts were visible between the malignant masses.

Case 4

The patient was a white woman, 60 years old, having cirrhosis of the liver and severe anemia. At necropsy (no. 5035) general peritonitis due to a ruptured duodenal ulcer was found. The liver was hobnailed and the inferior surface was attached to the head of the pancreas and the surrounding retroperitoneal tissues by dense, hard, white tissue. The head of the pancreas contained a carcinomatous mass which infiltrated the surrounding tissues and extended directly into the liver. In addition there were numerous very small metastases scattered throughout the liver and extensive involvement of the aortic lymph nodes.

Case 5

The patient was a white man, 82 years old, who was under observation on two occasions, presenting the symptomatology of hepatic cirrhosis with recurring ascites. Necropsy (no. 5537) revealed carcinoma of the stomach and metastases to the pancreas, adrenals and liver. The liver weighed 800 gm. and showed a typical Laënnec's cirrhosis. The metastases were present both in the fibrous portions and replacing hepatic parenchyma.

Case 6

The patient was a white man, 64 years of age, a chronic alcoholic, with carcinoma of the esophagus. Necropsy (no. 5696) revealed squamous-celled carcinoma of the esophagus with extensive thoracic and abdominal lymphatic metastases, and metastases to the omentum and liver. The liver weighed 2150 gm. and showed a typical Laënnec's cirrhosis and squamous-celled metastases replacing hepatic nodules.

Discussion

The rarity of the occurrence of metastatic malignancy in hepatic cirrhosis has been noted by several observers (Hanot and Gilbert,[1] White,[3] Rolleston and McNee[6]). Colwell[5] found only two cases in a series of 2634 autopsies upon patients with malignant disease performed at the Middlesex Hospital between 1854 and 1900. At City Hospital, Welfare Island, 6036 autopsies were performed between May

11, 1911, and December 31, 1940. Of these, 782 were upon patients with malignancy among whom there were 612 with carcinoma, exclusive of 25 examples of primary carcinoma of the liver. The six cases reported in this communication were the sole instances of metastases to cirrhotic livers.

The infrequent occurrence of hepatic cirrhosis in carcinoma of other organs offers one explanation for the rarity of metastases of the liver. Rolleston and McNee [6] noted that with intra-abdominal cancer, cirrhosis was unusual. In Colwell's [5] series, there were 27 instances divided as follows:

Males	*Females*
Mouth and face, 6	Breast, 6
Stomach, 3	Uterus, 6
Orbit, 1	Colon, 1
Testis, 1	
Penis, 1	
Kidney, 1	
Inguinal gland, 1	

In our series there were 22 instances of extrahepatic carcinoma with cirrhosis. The primary sites were as follows:

Kidney, 1	Lung, 1
Duodenum or ampulla, 4	Ovary, 1
Skin of neck, 1	Descending colon, 1
Tongue, 2	Splenic flexure, 1
Stomach, 5	Rectum, 1
Pancreas, 3	Esophagus, 1

Another explanation which may be advanced is that the cirrhotic liver is an unfavorable soil for growth of transplanted tumor cells. The predilection for secondary growths to involve the liver is well recognized. Willis [7] found metastases in 36 per cent of 323 patients with carcinoma upon whom he performed very careful autopsies. He also quoted an incidence of 33 per cent in a summary of data from Colwell,[5] Rolleston and McNee,[6] Kaufmann [8] and Kettle.[9] In our series of 612 cases of carcinoma, hepatic involvement occurred in one-third. Among 27 cirrhotic livers, however, Colwell found only 2 instances, or 7.4 per cent. We found metastases in 6 of 22 cirrhotic livers, a percentage of 27.2. This figure is somewhat lower than that for the entire series of carcinoma but within the range found by other observers (Kettle). It was of interest to note that of the 5 cases of gastric cancer with cirrhosis, 2, or 40 per cent, had metastases. This incidence is almost the same as that found by Willis (44 per cent) in the gastric malignancies in his series. However, since the material is so scanty, figures based on it must be taken with great reserve. If the cirrhotic liver *per se* were an unfavorable soil, one might expect to find cancerous emboli undergoing degeneration and not producing metastases. In

the 18 non-malignant cirrhotic livers such an observation was not made.

The method of involvement of the cirrhotic liver by carcinoma appears to be primarily hematogenous and does not differ from that of the normal liver. However, lymphatic metastasis or permeation is strongly suggested in cases 1 and 4. As Willis [7] has noted, once the liver is affected by secondary growth, the route by which the implant reached the organ is difficult to identify. There was a single example of invasion by contiguity.

The clinical course offers little aid in the diagnosis of hepatic involvement. In three cases (cases 2, 3 and 6) the malignant disease determined the syndrome. In two others (cases 4 and 5) the cirrhosis produced the predominant symptoms. In the cases of Poulain [2] and of Achard and Laubry,[4] the early symptoms were those of cirrhosis, the late symptoms being due to the malignancy.

Summary and Conclusions

Six cases of metastatic carcinoma affecting cirrhotic livers are reported. Only five other cases of metastatic malignancy in cirrhosis were found in the literature. The occurrence of extrahepatic malignancy and hepatic cirrhosis in the same case is uncommon. The liver may be involved by the hematogenous or lymphogenous routes or by direct contiguity. The rarity of metastatic malignancy in a cirrhotic liver may be due either to the unusual association of extrahepatic cancer and cirrhosis, or because the cirrhotic liver is an unfavorable soil for growth of transplanted tumor cells. The clinical course offers little aid in the diagnosis of hepatic metastases.

REFERENCES

1. Hanot, V., and Gilbert, A. Études sur les Maladies du Foie. Asselin & Houzeau, Paris, 1888, p. 13.
2. Poulain, A. Cancer de l'estomac. Noyaux secondaires développés dans un foie cirrhotique. *Bull. Soc. anat. de Paris,* 1899, **1**, 1089–1093.
3. White, W. H. Tumours of the Liver. In: Allbutt, T. C. A System of Medicine. The Macmillan Co., New York, 1897, **4**, 208. (Also: ed. 2, 1908, **4**, 217.)
4. Achard, Ch., and Laubry, Ch. Ascite lactescente. Cancer du côlon et cancer secondaire du foie atteint de cirrhose alcoolique hypertrophique. *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1902, **19**, 335–340.
5. Colwell, H. Malignant disease of the liver and bile passages. *Arch. Middlesex Hosp., London,* 1905, **5**, 123–141.
6. Rolleston, H. D., and McNee, J. W. Diseases of the Liver, Gall-Bladder and Bile Ducts. Macmillan & Co., Ltd., London, 1929, ed. 3.
7. Willis, R. A. The Spread of Tumours in the Human Body. J. & A. Churchill, London, 1934.
8. Kaufmann. (Quoted by Willis.)
9. Kettle, E. H. (Quoted by Willis.)

NEUROBLASTOMA, GANGLIONEUROMA AND FIBRONEUROMA IN A STILLBORN FETUS *

EDITH L. POTTER, M.D., and JOHN M. PARRISH, M.D.
(From the Department of Obstetrics and Gynecology, the University of Chicago and the Chicago Lying-in Hospital, Chicago, Ill.)

Recently, at the Chicago Lying-in Hospital, a stillborn fetus was delivered which exhibited a remarkable neoplastic involvement of the sympathetic nervous system and a generalized hyperplasia of the spinal nerves. The wide dissemination of tumor tissue, the great variation of cellular pattern and the location in certain regions where tumors of sympathetic origin have not been previously described (urinary bladder) make the case of unusual interest. The neoplastic growths include three varieties of tumors which are usually found arising individually in the spinal or sympathetic nervous system; they are neuroblastoma, ganglioneuroma, fibroneuroma.

REPORT OF CASE

Maternal History

Mrs. K., a primipara, 28 years old, had a normal prenatal course until the 27th week at which time she developed a progressive polyhydramnios. She was admitted to the hospital at the end of the 28th week and shortly thereafter went naturally into labor. Presentation was by breech and delivery progressed normally until difficulty was encountered in attempting to deliver the head. A diagnosis of hydrocephalus was made, a craniotomy was performed and the head was easily delivered following evacuation of the cranial contents. The subsequent maternal course was uneventful.

Gross Postmortem Examination

External Surface. The body (no. FA-38-568) was that of a white male infant weighing 1385 gm. and having a crown-heel length of 37.5 cm., a crown-rump length of 26.5 cm. The skin was normal except for postmortem discoloration. The second finger on each hand showed a contracture of the first phalangeal joint due to shortening of the flexor tendons. Both feet were clubbed and were in an equinovarus position.

Head. The head was large in proportion to the rest of the body but the fontanelles were of normal width and the bones of the calvarium seemed of normal thickness. A craniotomy had been performed and practically all of the brain tissue had been washed away. The base of the skull appeared normal except for slight flattening of the fossae. The buccal, pharyngeal and nasal cavities were normal.

* Received for publication, March 24, 1941.

Neck. The trachea was normal. The thyroid lay in its usual position and was entirely normal in appearance. There were several nodular masses of neoplastic tissue lateral and partially anterior to the trachea. The largest involved the vagus nerve on the left side of the neck. Immediately below its exit from the skull the vagus nerve swelled into a bulbous tumor mass slightly more than 1 cm. in diameter. Inferiorly this became fusiform and merged with the hyperplastic nerve trunk. On the left, behind the vagus nerve and the great vessels of the neck, was another similar round mass from which numerous nerve fibers extended. Two tumor nodules were also present on the right and were similar to those on the left except that they were somewhat smaller.

Thorax. All of the organs in the thoracic cavity were displaced upward as a result of subdiaphragmatic pressure, and were compressed against the anterior thoracic wall by the great enlargement of the spinal sympathetic ganglia in the posterior part of the chest. The ganglia extending along both sides of the vertebral column had undergone a neoplastic change and were part of an almost continuous mass of tumor tissue which involved all of the ganglia from the upper cervical to the lower sacral region. This nodular cordlike mass averaged over 1 cm. in diameter. The heart, lungs and thymus were normal except for changes in shape and location caused by compression from the tumors.

Abdomen. The abdominal viscera were displaced upward and anteriorly as a result of pressure exerted by a large tumor of the urinary bladder and the tumor masses in the posterior part of the abdomen. Extending along both sides of the spinal column was the neoplastic involvement of the sympathetic ganglia previously described. In addition there were two large tumors lying behind, but confluent with, the adrenal glands, the one on the left measuring 6 by 6 by 4.5 cm., the one on the right, 5 by 4.5 by 3 cm. They were connected across the midline in front of the aorta by similar tissue. There were also numerous small nodules throughout the abdominal cavity. The tumors in the region of the adrenals were light gray with numerous irregular scattered areas of hemorrhage and calcification. Those in the sympathetic chain were lighter in color, more uniform in appearance and contained much less calcium. A tumor of a third type was found in the bladder wall. It was of firmer consistency and grossly seemed to be composed of nerve fibers which were divided into bundles by intervening connective-tissue fasciculi. This tumor was confluent with the posterior wall of the bladder but did not invade the mucosa. It extended to the umbilicus above, posteriorly encircled the sigmoid colon

and rectum and finally terminated in the hypogastric plexus. It measured 4 by 6 by 6 cm.

The liver occupied the upper abdomen and was normal in size and shape. Immediately beneath the capsule and scattered throughout the substance were innumerable, small, pale yellow areas which appeared to be minute foci of tumorous tissue. The gallbladder and bile ducts were normal. The spleen appeared normal and contained no visible tumor tissue. The gastro-intestinal tract was normal throughout its entire length. In the mesentery of both the large and the small bowel there were many extremely hypertrophied nerves. The pancreas was normal although numerous extremely large nerves traversed the upper border. Both kidneys were flattened in the anteroposterior diameter from pressure exerted by the tumors involving the adrenal glands. A moderate degree of bilateral hydronephrosis was present although the ureters were of normal caliber. The testicles were undescended but appeared normal.

Microscopic Examination

Lung. The alveoli exhibited the pattern which is normal for a fetus which has never breathed. The only pulmonary abnormality consisted in the presence of a small group of neoplastic cells in an interlobular space at the level through which one section was taken. No point of attachment was visible.

Liver. The hepatic cells were well preserved and appeared normal. Diffuse extensive erythropoiesis was present. In all sections examined there were groups of tumor cells scattered throughout the parenchyma which appeared to be proliferating locally. There was no uniformity in distribution in relation to the architecture of the lobules. The cells had round, dark nuclei, pale cytoplasm and the cell boundaries were poorly demarcated. They were similar in character to the sympathicoblasts found in the adrenal tumors.

Pancreas. The islands of Langerhans, acini, and general pattern of the pancreas were normal. The nerves in its substance were increased in size and were distinctly abnormal in form, the central portions being made up of relatively acellular edematous tissue, with only the periphery showing the usual compactness of structure.

Spleen, kidneys, cardiac muscle, thymus, thyroid, testicles and *hypophysis* were normal.

Tumor of the Vagus Nerve. This tissue was an irregular mixture of cells in all stages of differentiation from sympathogonia to mature ganglion cells. Interspersed were large irregular areas of fine fibers arranged in interlacing bundles. They were pink with a hematocytic

eosin stain, blue with azo-carmine and black with silver stains. Very few nuclei were present in these areas. The fibers were similar histologically and in staining reaction to those derived from the sheath of Schwann. In a thin band around the periphery of the mass the cells and fibers were almost entirely mature and the appearance suggested that they may have been the original cells of the ganglia, with only the central areas comprising the neoplasm. The nodule was retort-shaped, the arm of the retort becoming continuous with the inferior portion of the nerve. This part was composed largely of fine fibers similar to those described above.

The other *tumors of the sympathetic ganglia* varied in structure. Grossly they ranged from a pale creamy white to deep gray, and histologically the light-colored ones had in general the same appearance as the nodule on the vagus nerve, while the darker ones bore more resemblance to the masses in the region of the adrenals. The latter contained large groups of sympathogonia with interlacing areas of fine fibrils. Although ganglion cells in all stages of differentiation were present, they were proportionately fewer than in the lighter colored tumors.

Adrenal Tumors. The masses in the region of the adrenals, those forming the connection across the midline, parts of the enlarged sympathetic chain and scattered nodules in the abdomen had a similar structure. They were composed of cells having a fairly uniform pattern, indistinguishable cell boundaries, and containing large, round nuclei showing coarsely distributed chromatin. They were divided into islands by bands of cells containing a large amount of fibrillar material, cells which resembled those of the sheath of Schwann. In some areas there was a tendency for the sympathicoblasts to attempt differentiation into ganglion cells, and in some places calcification and necrosis had occurred. The adrenal glands were still distinct structures although masses of tumor cells were present in the cortex and in the central portions. It is impossible to be certain whether the tumors arose within the glands, whether the cells within them were secondary growths, or whether all developed at the same time.

Bladder Tumor. This bore a close resemblance to a typical neurofibroma. It was composed almost entirely of fibers which were divided into large fasciculi by intervening masses of connective tissue. A few pale, ovoid nuclei were scattered among the fibers and the general appearance was similar to those tumors described as neurofibromas. In a few areas there were very small isolated groups of ganglion cells in various stages of differentiation.

Peripheral Nerves. All of the nerves in the body, both sympathetic

and spinal, were increased in size. There was no increase in neurons and possibly even a decrease, but the hypertrophy was due to a generalized increase in Schwann cells and to edematous, relatively acellular, areas.

Discussion

Tumors of the sympathetic nervous system have been recognized since the middle of the last century. The first group to be studied were the ganglioneuromas, tumors composed of mature ganglion cells and nerve fibers. Since the cells are similar in appearance to those normally found in sympathetic ganglia, a nervous origin for these tumors was quickly accepted. The tumors may be located in any part of the central, peripheral or sympathetic nervous system, or the adrenal gland, but they are found in the region of the paravertebral sympathetic ganglia more commonly than in other locations.

Neuroblastomas, adrenal neoplasms composed of small round cells with deeply staining nuclei, had been commonly regarded as sarcomas until Wright [1] (1910) summarized the literature concerning these tumors and stated the reasons for believing that they originate from cells of the embryonic sympathetic nervous system. Since then, many similar tumors have been observed and approximately 300 have been formally reported as neuroblastomas.

Pheochromocytomas most frequently originate in the adrenal gland but are considerably less common than neuroblastomas or ganglioneuromas. They also may be found in sympathetic ganglia or any place where chromaffin tissue normally occurs. The tumors are composed of mature chromaffin cells and the hormone normally produced by the adrenal medulla has been extracted from them.

Neurofibromas, although usually not classified with tumors of the sympathetic system, may arise from sympathetic nerves as well as from those of the cerebrospinal system and consist of a neoplastic proliferation of Schwann cells. Since Schwann cells, like ganglion cells and chromaffin cells, have differentiated from migratory cells from the neural crest, there is a close interrelationship of all three.

The origin and varying degrees of differentiation found in tumors of sympathetic nervous systems are easily explained on the basis of embryologic development. During the early growth of the embryo, cells migrate from the neural crest to various parts of the organism where they become permanently located and develop into sympathetic ganglia, Schwann cells or chromaffin tissue. These primitive cells are rarely seen in ganglia after the first few weeks of intra-uterine development but may be found in the adrenal cortex throughout fetal life

and may occasionally persist after birth. The fact that cells in this location undergo morphologic and physiologic differentiation later than the other cells of the nervous system may be a contributing factor to the frequent localization of tumors composed of primitive nerve cells in the adrenal glands.

At the time of migration from the neural crest, sympathetic nerve cells are histologically similar to those of the central nervous system and differ only in potentiality for future development. They are small cells whose hyperchromatic nuclei are surrounded by a thin layer of cytoplasm, or are enmeshed in a cytoplasmic mass whose individual cell boundaries cannot be distinguished. Tumors composed of such cells are known as neuroblastomas or more accurately as sympathogoniomas, sympathogonia being the term for entirely undifferentiated sympathetic cells.

Sympathogonia, in the normal process of growth, develop larger, slightly vesicular nuclei, the chromatin becomes clumped, nucleoli appear, and the cytoplasm increases in amount. The cells in this stage are called sympathicoblasts and the tumors composed of such cells, sympathicoblastomas. The term neuroblastoma, as usually used, includes both groups of tumors. Although few fibers are seen under normal conditions at this stage of differentiation, large masses of fibers may be seen in both sympathogoniomas and sympathicoblastomas.

In further development the sympathicoblasts follow one of several possible courses; part become the chromaffin cells, part become the ganglion cells and part develop into Schwann cells. In neoplasms arising from sympathicoblasts, similar lines of differentiation may be followed and the cells may mature into ganglion, Schwann or chromaffin cells. Tumors containing these cells are known as ganglioneuromas, neurofibromas and pheochromocytomas, respectively. All exhibit an extremely variable pattern as a result of the differences in degree of maturation attained in individual tumors, and also as a result of the difference in the relative proportions of cells and fibers which are present.

One would expect that a mixture of ganglion and pheochrome cells would occasionally be found in the same tumor, but this appears to occur very rarely. One of the few instances in which neuroblasts, ganglion cells and chromaffin tissue were all present was reported by Wahl[2] (1914) and was located in the mesentery near the left kidney. Other combinations which have been described are neurofibromas and ganglioneuromas, neurofibromas and pheochromocytomas and, most commonly of all, neuroblastomas and ganglioneuromas.

In the fetus reported in this paper the tumors exhibit an inter-

mixture of cells which vary from the most primitive type of neuroblast to mature ganglion cells and from areas composed largely of ganglion cells and containing relatively few fibers to others in which ganglion cells are completely absent and only fibers and Schwann cells are visible. The tumors are much more widely disseminated and there is a much more complete involvement of the sympathetic nervous system than has previously been observed. The large fibroneuroma of the bladder is also unique. Due to the method of original fixation it is impossible to be certain whether or not there is differentiation into chromaffin cells. Although this fetus was delivered 2 months prematurely, necrosis and calcification of the tumors had already occurred.

The miliary dissemination of neuroblastic tissue throughout the liver may probably be considered a result of the same stimulus which produced neoplastic changes in the rest of the sympathetic nervous system. According to Maximow [3] (1930), collections of cells arising from sympathogonia and giving a chromaffin reaction are normally found in this organ. Most authors have considered the frequent association of hepatic tumors with adrenal neuroblastomas a result of metastases from adrenal tumor to liver. This may be a reasonable assumption in the presence of a single primary tumor. In the present case, however, stimulation of the cells normally distributed throughout the liver, simultaneous with the stimulation of the rest of the sympathetic nervous system, seems a more probable explanation of the origin of these tumor masses.

This case exemplifies most graphically the close interrelationship of ganglioneuromas, neuroblastomas and fibroneuromas and the common identity of the stimulus required for their production.

Summary

A premature stillborn fetus is described in which there was a generalized neoplasia involving all of the sympathetic ganglia, the adrenal glands and the wall of the urinary bladder. Multiple tumor foci were present in the liver. The sympathetic and spinal nerves were hypertrophied.

It exemplifies the close relationship which exists between neuroblastomas, ganglioneuromas and fibroneuromas.

Note: The authors are deeply indebted to Percival Bailey for aid and advice.

References

1. Wright, J. H. Neurocytoma or neuroblastoma, a kind of tumor not generally recognized. *J. Exper. Med.*, 1910, **12**, 556–561.

2. Wahl, H. R. Neuroblastoma: with a study of a case illustrating the three types that arise from the sympathetic system. *J. M. Research*, 1914, **30**, 205–260.

3. Maximow, A. A. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1930.

ADDITIONAL BIBLIOGRAPHY

Dalton, Norman. Infiltrating growth in liver and suprarenal capsule. *Tr. Path. Soc. London,* 1885, **36,** 247–251.

Lorentz, Wilh. Ein Fall von gangliösem Neurom (Gangliom). *Virchows Arch. f. path. Anat.,* 1870, **49,** 435–437.

Manasse, Paul. Zur Histologie und Histogenese der primären Nierengeschwülst. *Virchows Arch. f. path. Anat.,* 1896, **145,** 113–157.

McFarland, Joseph, and Sappington, S. W. A ganglioneuroma in the neck of a child. *Am. J. Path.,* 1935, **11,** 429–448.

Miller, J. W. Ein Fall von metastasierendem Ganglioneurom. *Virchows Arch. f. path. Anat.,* 1908, **191,** 411–421.

Robertson, H. E. Das Ganglioneuroblastom, ein besonderer Typus im System der Neurome. *Virchows Arch. f. path. Anat.,* 1915, **220,** 147–168.

von Fischer, R. F. Zur Kenntnis der Neurome des Sympathikus. *Frankfurt. Ztschr. f. Path.,* 1922, **28,** 603–628.

Wiesel, Josef. Bemerkungen zu der Arbeit H. Küsters "Über Gliome der Nebennieren." *Virchows Arch. f. path. Anat.,* 1905, **180,** 553–555.

DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Anterior view of the abdominal and thoracic viscera after removal of the liver. The two tumor masses in the region of the adrenal glands and the contiguous mass in the midline have forced the kidneys downward and have distorted their contour. The tumor of the bladder is visible below the other organs. A = adrenal gland visible on surface of one of tumor masses, T. B = bladder tumor. L = lungs. H = heart. K = kidneys.

FIG. 2. The thoracic and abdominal viscera have been drawn to the right revealing the numerous tumor nodules in the region of the sympathetic ganglia and along the nerve trunks. The generalized hypertrophy of nerve trunks is also well shown.

Potter and Parrish Neuroblastomas in Stillborn Fetus

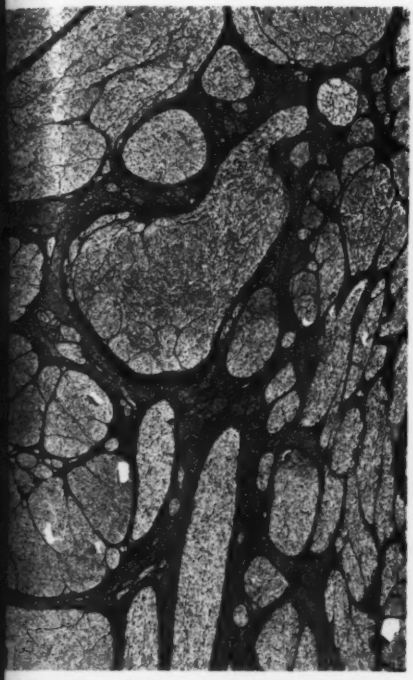
PLATE 21

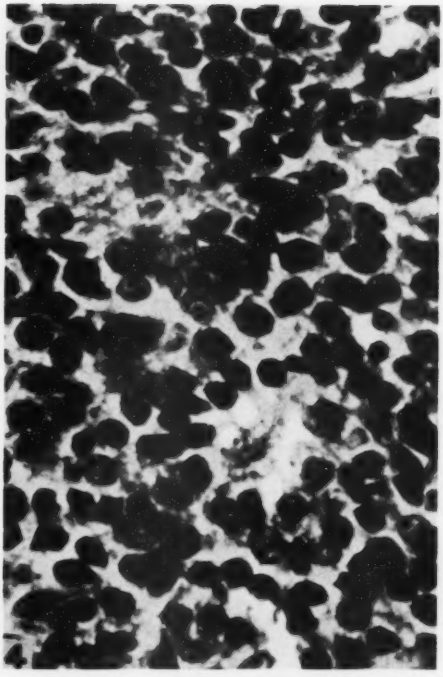
FIG. 3. Tumor from the bladder showing separation of neoplastic fibers into interlacing bundles by intervening connective tissue. No ganglionic or neuroblastic cells are present. Hematoxylin and eosin stain. × 16.

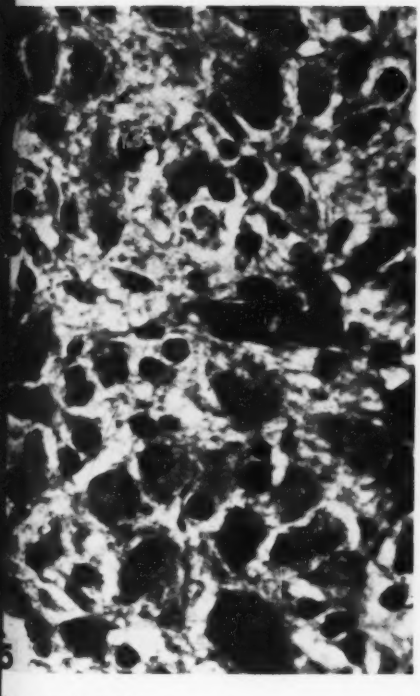
FIG. 4. Sympathicoblasts in one of the tumors of the thoracic sympathetic ganglia. Similar areas were present in all tumors except that in the urinary bladder. Hematoxylin and eosin stain. × 612.

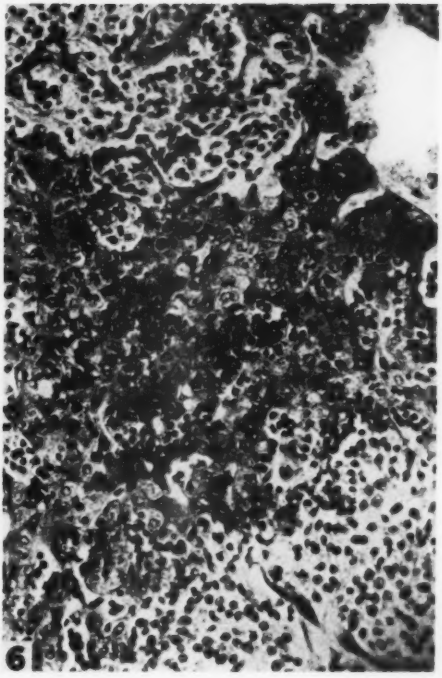
FIG. 5. Immature ganglion cells from one of the adrenal tumors. Similar cells were found in all tumors. In the bladder they were isolated in very small areas and comprised only a small fraction of the entire tumor. Hematoxylin and eosin stain. × 612.

FIG. 6. Tumor nodules in the liver. The light-staining cells are sympathicoblasts; the darker ones are hepatic cells. The latter are interspersed with numerous normal areas of erythropoiesis. Hematoxylin and eosin stain. × 197.









ICTERUS OF THE ADULT BRAIN *

Report of a Case

Enid K. Rutledge, M.D., and Karl T. Neubuerger, M.D.
(From the Departments of Pathology and Neuropathology, University of Colorado School of Medicine and Hospitals, Denver, Colo.)

Bile pigments in the brain, to our knowledge, have never been found in adults, even in patients with severe jaundice showing marked biliary pigmentation of dura and leptomeninges. In children, however, bile pigments are observed in certain areas of the brain in the rare condition of Kernikterus. Surveys of the literature on Kernikterus are given in the recent papers of Zimmerman and Yannet,[1] and of Fitz Gerald, Greenfield and Kounine.[2] Broman,[3] in 1940, stressed the fact that bile pigment is never found in the brains of adults; the blood-brain barrier in adults is sufficiently impervious to prevent the transfer of bile from the blood vessels into the brain tissue. In view of these conclusions, we believe that observations made in the study of a recent case showing extreme jaundice are worthy of record. The brain in this case showed no changes comparable to that of Kernikterus, namely, pigmentation of circumscribed nuclear masses, but did reveal, in the cortex, several yellowish green foci containing bile pigment.

Report of Case

F. G. (C. G. H. no. 48629), a white American-born housewife, 74 years old, was admitted to the Colorado General Hospital on November 27, 1940, with the complaints of progressively deepening, painless jaundice with severe itching for 9 months, intolerance to fatty foods with associated gastric discomfort, nausea and vomiting for 2 or 3 months, burning and tingling sensations in the feet for 3 months and, for 2 months, an almost continuous desire to sleep. The patient had lost 25 pounds during the 9 months, was extremely constipated, had clay-colored stools and passed dark brownish yellow urine, with burning on urination and nocturia. Physical examination revealed an emaciated, elderly woman with a deep yellow, dry skin and marked abdominal distention. Temperature upon admission was 98.2° F.; respiration rate was 24 and pulse rate, 84 per minute; blood pressure was 120/60. Urinalysis findings were: specific gravity, 1.023; albumin, 2 plus; pus cells, 2 plus. Blood studies revealed: hemoglobin content, 11 gm. per 100 cc.; red blood cells, 3,710,000; white blood cells, 17,700 with 89 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes and 1 per cent endothelials; sedimentation rate of 32 per cent in 30, and 55 per cent in 60 minutes; prothrombin time of 87 per cent of normal; icteric index of 150 and direct van den Bergh reaction. There were 19 mg. of bilirubin, 34 mg. of nonprotein nitrogen and 88 mg. of sugar per 100 cc. of blood. Roentgenogram of the chest revealed no intrapulmonic pathologic changes. Histologic examination of ascitic fluid by sectioning sediment blocked after centrifugation failed to show tumor cells. On the second hospital day, 4700 cc. of dark brown, ascitic fluid were removed from

* Received for publication, May 12, 1941.

the body, permitting the palpation of a mass in the upper portion of the abdomen. Clinical diagnosis of advanced carcinoma of the head of the pancreas was made. The clinical course was progressively downhill. Throughout most of the hospital stay the patient was stuporous. On December 6th she was transferred to a nursing home where she remained, under medical care, in a stuporous state until her death on December 26th.

Postmortem examination of the extremely jaundiced body showed a mucinous adenocarcinoma of the gallbladder. The gallbladder was small, thick-walled and contained numerous mixed pigment-cholesterol calculi. Neoplastic tissue involved the portal lymphatic system with obstruction of the common bile duct, extension along the main intrahepatic ducts, secondary cholangiectasis, suppurative choledochitis, cholangitis, associated with scattered small abscesses and focal necrosis of the liver. Other findings included suppurative cystitis and slight pyelonephritis, marked atherosclerosis of the aorta, with ulceration and calcification, and atherosclerosis of the coronary and cerebral arteries. A detailed description of the changes in the brain follows.

Gross Examination of the Brain

At necropsy the leptomeninges appeared deeply yellow-tinged and over the left occipital region presented several greenish yellow spots, 2 by 1 cm. in maximum area. In the thin walls of the main and smaller arteries at the base of the brain were numerous atheromatous plaques.

After 24 hours of formaldehyde fixation, the structures of the cortex appeared well preserved. No tumor tissue was evident macroscopically in frontal sections through the brain. In a rather large area in the right occipital lobe, conspicuous foci with a greenish discoloration were disclosed. Most of these foci were deepset in the convolutions near the sulci; the majority were limited to the cortex but a few involved the adjoining white matter. Some foci appeared as spots a few millimeters in diameter; others, as narrow stripes. In the depth of the white matter of the right cerebellar hemisphere was a small yellow focus. This was 7 by 5 by 3 mm. in size, was irregularly outlined and presented homogeneous yellow surfaces on section. Macroscopically, the brain otherwise appeared normal.

It was possible to extract a sufficient amount of the pigment with chloroform to carry out the Gmelin reaction on the crude extract; this reaction was positive. It was not possible to obtain sufficient pigment for more exact chemical identification.

Microscopic Description of the Brain

There was no evidence of metastatic neoplasm in any section of the brain tissue. The focus in the white matter of the cerebellum was surrounded by a zone of loose, edematous tissue in which the ground

substance was vacuolated and appeared reticular. The focus itself was richly vascularized: many of the vessels were dilated and some were lined by swollen endothelial cells. The tissue appeared less reticular and vacuolated than that of the surrounding zone and contained residua of broken-down myelinated fibers and scattered swollen astrocytes. It was composed chiefly of loosely arranged scavenger cells. These scavenger cells were round and somewhat variable in size; they had one or two moderately large nuclei and a granular or vacuolated cytoplasm. Several gitter cells appeared diffusely yellow-tinged, although no granular bile pigment was apparent in this focus. The area thus appeared as a focus of softening, with slight, diffuse, biliary pigmentation in some of the gitter cells but no pigmentation of the interstitial tissue. The grossly visible generalized pigmentation, not discernible microscopically, was probably the result of a diffuse imbibition with bile pigment.

The other foci were in part similar to that in the cerebellum. They showed features of softening and were surrounded by vacuolated edematous tissue. On the other hand, they contained a number of scavenger cells in which there was much more bile pigment, either diffuse or in granules of variable size with indistinct borders. In the same area, swollen astrocytes failed to display stored bile pigment. In some areas the "softening" was composed partially of a vascular syncytial tissue, the cytoplasm of which appeared clear and faintly foamy, suggestive of pseudoxanthomatous granulations. Furthermore, in other amorphous, apparently necrotic areas there were deposits of calcified material, in small granules, partially coalesced or in elongated, more or less tortuous strands, probably formed from calcified, necrotic myelinated fibers. In yet other areas, there were clusters of dense aggregates of calcified fibers and globules. Several intracortical vessels were surrounded by thick collars of calcified granules. The softened areas were located in the subcortical white matter and showed a few irregularly scattered, calcified fibers. The main calcified zone involved the deep cortical layers. The upper and middle layers of the cortex showed recent necrosis; the second layer contained many calcified nerve cells. In this area the supporting tissue showed little reaction. On application of the Nissl stain this necrotic zone clearly exhibited bile pigment. This pigment, yellowish brown and granular, was present within both scavenger and necrotic, ghostlike nerve cells, as well as in intramural cells of the vessels. In addition, the involved cells, especially the nerve cells, were diffusely pigmented. Numerous necrotic nerve cells appeared deeply eosinophilic, as is frequently evident in ischemic necrosis. In other areas in the cortex there were scattered petechiae.

Fat stain revealed the presence of lipoids in the scavenger cells and demonstrated clearly the development of such cells from progressive microglia cells. By means of a silver stain, swollen astrocytes, especially of fibrillary type, were found to be prominent in the tissue surrounding the foci. Gram-picric stain of various sections revealed no evidence of bacteria. Staining for iron was negative. Von Kossa's stain for calcium was strongly positive.

Several small arterial branches in the meninges near the foci presented mural atherosclerotic plaques, which narrowed but did not occlude the lumina. There were no thrombi. Numerous intracortical arterioles were markedly sclerosed; the thickened walls compressed the lumina. The meninges were free from inflammatory changes.

Discussion

The foci described above showed in part the features of ordinary softening as seen in arteriosclerosis. In addition they contained areas of necrosis with marked calcification. Bile pigment was present in necrotic nerve cells, scavenger cells and endothelial cells. We believe that the blood-brain barrier, which is probably located in the vascular intima (Broman,[3] Spatz[4]) became pervious to bile within these foci. It is to be concluded from the papers of these authors that trypan blue, which under normal conditions does not enter the nervous parenchyma, can do so if there is a local disturbance in the hemato-encephalic barrier. This is probably likewise true of bilirubin. A disturbance in that barrier may well be assumed to develop in the capillaries within a focus of arteriosclerotic or traumatic necrosis. As in experiments with trypan blue and in Kernikterus, necrotic tissue elements, particularly necrotic nerve cells, seem to have a certain affinity for the dye. The ensuing distribution and location of the pigment within the tissue constituents, as indicated above, are essentially consistent with the findings in Kernikterus. Normal nervous parenchyma has a lack of affinity for trypan blue (King[5]) and obviously for bile pigments.

The complete necrosis and calcification in certain areas are not readily explained. The deficient reaction of the local supporting tissues may be due, of course, to the time factor, inasmuch as the necrosis could have been of quite recent origin, with death occurring before the reactive changes set in. On the other hand, we may assume a severe toxic activity of the penetrating bile, necrotizing not only the nervous parenchyma but also the supporting tissue. A lengthy discussion of the causes of calcification in necrotic areas of the brain is beyond the scope of this paper. The local colloids possibly undergo a change so as to behave like gels; the latter display a certain affinity for calcium salts (Meyer,[6] Eaton, Camp and Love[7]).

The change was less marked in the white matter of the cerebellum than in the gray matter of the occipital lobes. This is consistent with the fact stressed by Spatz [4] that quite generally the gray matter stores vital dyes more intensely than the white matter, the former having a better vascular supply.

Although findings similar to those discussed in this paper, to our knowledge, have never been described, we are of the opinion that they may be found in patients showing a combination of extreme jaundice and cerebral arteriosclerosis.

Experimentally, it should be possible to produce obstructive jaundice of long duration and then, by traumatization of the tissue or by ligation of an arterial branch, to produce foci of brain softening. If our view is correct, such areas should show bile pigment. This would be consistent with the experiments of Broman [3] and Spatz [4] with trypan blue (tingibility of nervous tissue in foci of traumatic destruction or embolic damage).

Summary

1. Foci of cerebral softening containing bile pigment are described as found in a patient with carcinoma of the gallbladder and severe generalized jaundice.

2. The pathogenesis of this condition is discussed.

Note: We are indebted to Bernard B. Longwell, of the Department of Biochemistry, University of Colorado School of Medicine, for making the chemical examination.

References

1. Zimmerman, H. M., and Yannet, Herman. Cerebral sequelae of icterus gravis neonatorum and their relation to Kernikterus. *Am. J. Dis. Child.*, 1935, **49**, 418–430.

2. Fitz Gerald, G. M.; Greenfield, J. G., and Kounine, B. Neurological sequelae of "Kernicterus." *Brain*, 1939, **62**, 292–310.

3. Broman, Tore. Über die Blut-Hirnschranke, ihre Bedeutung und ihre Beziehungen zur Blut-Liquorschranke. *Arch. f. Psychiat.*, 1940, **112**, 309–326.

4. Spatz, H. Die Bedeutung der vitalen Färbung für die Lehre vom Stoffaustausch zwischen dem Zentralnervensystem und dem übrigen Körper. *Arch. f. Psychiat.*, 1933, **101**, 267–358.

5. King, L. S. The hematoencephalic barrier. *Arch. Neurol. & Psychiat.*, 1939, **41**, 51–72.

6. Meyer, W. C. Beiträge zur Frage des Pseudokalkes im Zentralnervensystem. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1933, **146**, 393–411.

7. Eaton, L. M.; Camp, J. D., and Love, J. G. Symmetric cerebral calcification, particularly of the basal ganglia, demonstrable roentgenographically. *Arch. Neurol. & Psychiat.*, 1939, **41**, 921–942.

BILATERAL FUSIFORM ANEURYSMS OF THE CERVICAL PORTION OF THE INTERNAL CAROTID ARTERIES *

JOHN G. RIDDLER
(From the Department of Gross Anatomy, Tulane University, New Orleans, La.)

During the academic year 1939–1940, there was found in the anatomical laboratory of Tulane University a cadaver with bilateral fusiform aneurysms of the cervical portions of the internal carotid arteries. The subject was a negress about 50 years old. Other pathological conditions found in the body were: primary carcinoma of the head of the pancreas, cirrhosis of the liver and uterine leiomyomata.

Each aneurysm was located at the beginning of an internal carotid artery and each was 19 mm. in length with internal diameters of 8 mm. The internal diameter of the vessels above the dilatations was 5 mm. Microscopical sections were made through the aneurysms and through the arteries above and below the distended portions. One set of sections was stained with hematoxylin and eosin, one set with Weigert's elastic tissue stain and another with van Gieson's stain.

MICROSCOPICAL EXAMINATION

Aneurysmal Portion of Right Carotid Artery. Sections of the dilated portion of the right carotid artery, stained with hematoxylin and eosin, showed the endothelial lining virtually destroyed, possibly as the result of autolysis. The intima generally was much thickened and very irregular in outline, some areas being thrown up into definite elevations. It presented areas of atheromatous degeneration, some of which showed lime salt deposits and other amorphous structureless material. The media showed extensive degenerative changes of the same nature, with lime salt deposits and slight increase in connective tissue. In one area the wall of the vessel was very thin, being made up of a thin layer of intima and only the outer portion of the media and adventitia. In the adventitia were noted many groups of lymphocytes and plasma cells around the blood vessels. Sections stained by van Gieson's method showed a marked increase of the connective tissue element throughout, which had largely replaced the muscular structure. In one area the inner muscular layer had entirely disappeared and the wall of this part was made up only of the intimal covering and outer medial coat. With Weigert's elastic tissue stain the normal internal elastic lamina was not demonstrable as a relatively solid, wavy cord but the elastic fibers occurred as fine fibrils throughout the wall, being most marked

* Received for publication, June 5, 1941.

in the deeper portions of the media. In one area there was a definite rupture of these fibers and the overlying structure corresponded to one of the degenerating atheromatous layers of the inner portion of the media and intima. The outer medial coat showed many of the elastic tissue fibrils running in a tangential rather than in a circumferential manner. These changes were indicative of arteriosclerotic degeneration with lime salts deposited in some areas, resulting in rupture of the intima and medial coats and permitting dilatation of the vessel with formation of a fusiform aneurysm.

TEXT-FIGURE 1. Drawing showing the fusiform aneurysm of the cervical portion of the internal carotid artery on the right side. An identical lesion was present on the left side.

Non-dilated Portion of Right Carotid Artery. Sections from this region showed some slight thickening of the intimal coat which was rather irregular but presented only small areas of atheromatous degeneration. In the media there was a slight increase of connective tissue and also in the adventitia. Perivascular infiltration was demonstrated.

Left Carotid Artery. Sections from both aneurysmal and non-dilated portions showed similar pictures to those from the right, but atheromatous changes were not as marked.

Diagnosis. While there was slight perivascular infiltration, it is not believed that syphilis was the causal factor. A histological diagnosis of arteriosclerosis (atheroma), with softening of the intimal coat,

rupture of the medial coat and fusiform aneurysms was made. The aorta showed moderate atheromatous change with no evidence of luetic involvement either grossly or microscopically.

Discussion

I have been unable to find any report of bilateral aneurysms of the cervical portion of the internal carotid arteries. Only four reports of bilateral aneurysms of the carotid arteries were found in a survey of the available literature.

Temple (1891) described bilateral aneurysms of the common carotid arteries with involvement of the innominate artery on the right side.

A short description of Gay's case (1894) of ligation of both common carotid arteries for double aneurysm follows:

> A patient, 7 years of age, noticed a small, pulsating tumor on the left side of his neck about 1 inch above the clavicle. Ten years later he noticed a tumor the size of a chestnut on the right side of the neck at the level of the upper edge of the thyroid cartilage. Nine years later, at the time of the first operation, the tumor on the left side extended from a point 1½ inches above the clavicle to the mastoid region, filling the upper part of the anterior triangle and at the same time elevating and pushing the sternomastoid muscle backwards. By this time the tumor on the right side was about 1 inch in diameter. Pressure on the common carotid arteries below the tumor stopped the pulsation in both aneurysms. Two ligatures were placed on the left common carotid artery 1½ inches above the clavicle and the artery divided between the ligatures. Ten months later the right common carotid artery was ligated and divided about 2 inches above the clavicle. Four months after the second operation a small pulsating tumor appeared in the center of the sac on the left side. This secondary aneurysm increased in size very slowly. Nineteen months after the second operation the patient was perfectly well except for the small secondary aneurysm which gave him no trouble.

Friedrich (1934) described a patient with exophthalmos and other symptoms which indicated an hypophyseal tumor. The autopsy disclosed an aneurysm of the cavernous portion of each internal carotid artery.

Bozzoli (1937) described bilateral aneurysms of the cavernous portions of the internal carotid arteries. Pressure of the dilatations on the pituitary gland caused an ocular syndrome and acromegalic signs.

Summary

An example of bilateral fusiform aneurysms of the cervical portions of the internal carotid arteries is described.

Note: The microscopical sections were examined by Dr. John A. Lanford, Department of Pathology and Bacteriology, Tulane University.

BIBLIOGRAPHY

Bozzoli, A. Aneurisma bilaterale della carotide interna. *Riv. oto-neuro-oftal.*, 1937, **14**, 304–316.

Friedrich, Günther. Traumatisches sackförmiges Aneurysma der rechten und linken Arteria carotis interna nach alter Oberkieferresektion. *Zentralbl. f. Chir.*, 1934, **61**, 1586–1592.

Gay, G. W. A case of ligation of both common carotid arteries for double aneurism. Recovery. *Med. & Surg. Rep. Bost. City Hosp.*, 1894, **5**, 101–107.

Temple, W. F. A case of aneurysm of each carotid, one involving the innominate. *Boston M. & S. J.*, 1891, **124**, 136.

TRYPAN-BLUE VITAL STAINING IN STUDIES OF VIRUS LESIONS ON CHORIO-ALLANTOIC MEMBRANES *

JEAN V. COOKE, M.D., AND RUSSELL J. BLATTNER, M.D.
(From the Department of Pediatrics, Washington University School of Medicine and the St. Louis Children's Hospital, St. Louis, Mo.)

In the cultivation of filtrable viruses on the chick chorio-allantois, the cellular proliferations produced by the virus on the membrane appear characteristically as discrete, semi-opaque gray foci. In the case of some viruses, such as variola and vaccinia, for example, these foci are relatively large and easily seen. On the other hand, with certain viruses, among which may be numbered encephalitis, influenza, yellow fever and lymphocytic choriomeningitis, the lesions produced are so small that all investigators have experienced difficulty in recognizing them. This is especially true under certain conditions, and when the foci are few in number. Obviously, lesions must reach a certain size to be visible even with a hand lens, and since they often measure less than 0.5 mm. when fully developed, scanty, scattered foci cannot be recognized. In this paper there is described a method for making these small focal lesions visible and readily recognizable by staining them with trypan-blue, and for making permanent mounts of such stained membranes for later, more careful examination.

Trypan-blue, after intravenous injection, has the property of being taken up and flocculated by certain living cells, notably reticulo-endothelium, and to a certain degree by other connective tissue elements. It stains only living cells, and does not produce a diffuse coloration of all tissue elements. So far as we are aware, the use of the dye for differentiation and demonstration of proliferative virus foci on egg membranes has not been described previously.

Our earlier observations on the effect of injecting trypan-blue into a vein of the chorio-allantois showed that almost all of the pigment was retained in the embryo, which became blue, but that the membrane itself remained unstained. However, if the trypan-blue solution was applied directly to the membrane containing virus lesions and the egg reincubated for a short time, it was found that the areas of focal proliferation became blue, while the remainder of the membrane was only faintly stained. In normal membranes of uninoculated eggs treated in the same manner, the cells take up only minute amounts of the stain. The membranes are pale blue and without focal intensifica-

* Received for publication, May 29, 1941.

tion. Virus-infected membranes, after being stained, removed from the egg and washed free of the excess stain, can be fixed flat in a solution of formaldehyde and mounted in glycerol jelly under a coverglass. This serves to "clear" the preparation and brings out more distinctly the differential staining of the focal lesions. Such mounted specimens are permanent and can be studied with the higher powers of the microscope. Details of the staining and mounting technic follow.

Method

When a membrane is ready for examination, 1 cc. of a 0.5 per cent aqueous suspension of trypan-blue is placed directly upon the membrane through the window in the shell. Good general distribution of the stain may be obtained by gentle rotation of the egg. More recently, however, we have found it more satisfactory to apply the stain by means of an atomizer (DeVilbiss no. 15), of which the tip may be directed downward. In this case no rotation is necessary. After staining, the shell window is closed and the egg placed in the incubator for from 10 to 30 minutes, depending on the age and extent of the lesions. More extensive lesions require a shorter time to take up the stain, and may become too intensely colored for satisfactory examination if the stain is left on too long.

The membrane is removed in the usual manner, washed gently in physiological saline solution to remove the excess of trypan-blue, and fixed flat in a 4 per cent solution of formaldehyde for a few minutes. After draining away the excess fluid, it is placed on a glass slide, 2 by 2.5 inches, and mounted in a glycerol-gelatin mixture consisting of 50 per cent glycerol, 5 per cent gelatin and 1 per cent phenol. The gelatin should be soaked in water for about 2 hours before adding the glycerol and phenol, then heated gently for a few minutes while stirring. For mounting the membrane, the glycerol jelly is melted and, while still warm (about 70° C.), is dropped on the membrane until it is well covered. The slide is flamed gently, but not enough to produce bubbles, and then mounted with a warmed coverslip. Gentle pressure is applied until the jelly has begun to set. When fully hardened the excess jelly is removed and the edges sealed. For this purpose clear Duco cement thinned with acetone is very satisfactory.

Discussion

The method outlined has been used routinely during the past year on several thousand membranes, and has been of considerable value in the recognition of virus lesions which could not have been identified without its use. While most of the observations have been made with St. Louis encephalitis virus, we have found that this method shows equally well the lesions of Japanese encephalitis, lymphocytic choriomeningitis, influenza, yellow fever, herpes simplex, vaccinia and variola. Normal membranes, and those to which clear broth or sterile serum had been added 1 to 2 days previously, when stained by trypan-blue and mounted, show none of the focal staining characteristic of virus lesions. Microscopic examination of these membranes reveals faintly stained ectodermal cells, occurring in small groups or sheets. Cells around areas of trauma may also appear stained. After a little

practice, however, no difficulty is experienced in identifying virus foci from non-specific or traumatic areas.

The recognition of lesions has been particularly helpful in studies of St. Louis encephalitis in which the foci produced are small. By the method described it is often possible to recognize the stained lesion as early as 18 hours after inoculation, and always after 24 hours. Even when the lesions are few and scattered, as with inoculation of 0.1 cc. of 10^{-7} dilution of the virus, they can be identified regularly as virus foci. Influenza virus foci, which are even smaller than those of St. Louis encephalitis, are clearly differentiated and recognized by the trypan-blue stain. In addition, the method has been valuable in the titration of virus dilutions, since scanty, scattered lesions are readily visible; and also in the study of tests of immune serum neutralization, in which the absence of foci, or the counting of the lesions, are important criteria.

Under low magnification the lesion appears as a discrete focal accumulation of proliferating cells stained blue, and in sharp contrast with the surrounding membrane. In older lesions some necrosis may be seen in the center, and some infiltration of inflammatory cells at the periphery. With higher magnification the proliferating cells contain many fine blue granules. Mounts of entire membranes are not very satisfactory for detailed cytological study because of their thickness, but after imbedding in paraffin, cross sections show the characteristic focal proliferation of ectodermal cells containing trypan-blue granules. As yet we have made no careful study of the appearance of these lesions in cross section.

The use of other stains, such as janus green and vital red, has been rather unsatisfactory since they produce a more general staining of the membrane, and differentiation of the foci is poor. While trypan-red is similar to trypan-blue, the chorio-allantois takes up the red stain more diffusely and identification of individual lesions is more difficult.

The permanent mounting of membranes bearing virus lesions has numerous advantages, facility is acquired readily and the mounts may be made routinely by technicians. The membranes may be thus preserved and filed, and the lesions studied by several observers at any later time. Actual membranes may be projected directly on a lantern screen for demonstration.

Summary

A method of staining virus foci on chick chorio-allantoic membranes by trypan-blue is described, with directions for clearing and mounting such membranes for permanent record and study. Its use facili-

tates greatly the recognition and enumeration of the lesions in the study of viruses and virus immunity in the developing egg.

NOTE: We wish to express our appreciation to Margaret G. Smith of the Department of Pathology of the Washington University School of Medicine, and to Edwin H. Lennette of the International Health Division of the Rockefeller Foundation, for furnishing viruses used in this study. Antoinette Wagenfuehr assisted in the technical procedures.

DESCRIPTION OF PLATE

PLATE 22

FIG. 1. Vaccinia (48 hours, dilution 10^{-6}). The lesions appear as large, discrete foci, standing out in sharp contrast to the normal membrane. While the large pocks of vaccinia may be seen readily on unstained membranes, this stained preparation emphasizes the striking differential staining reactions of virus-infected cells of the chorio-allantois. × 3.

FIG. 2. St. Louis encephalitis (72 hours, dilution 10^{-8}). Here is seen a somewhat diffuse, faint staining of the membrane on which more deeply stained focal areas of cellular proliferation stand out prominently. × 3.

FIG. 3. Herpes (72 hours, dilution 10^{-1}). Very numerous, deeply stained foci, for the most part discrete but confluent in certain areas. × 3.

FIG. 4. Herpes (72 hours, dilution 10^{-1}). An identical sister membrane unstained for comparison, but under slightly greater magnification. The herpetic foci appear merely as somewhat indefinite, hazy, poorly defined opacities. × 3.5.

FIG. 5. St. Louis encephalitis (same as in Fig. 2). Higher power of Figure 2 showing the sharp differentiation of the virus foci from the faintly stained membrane. × 35.

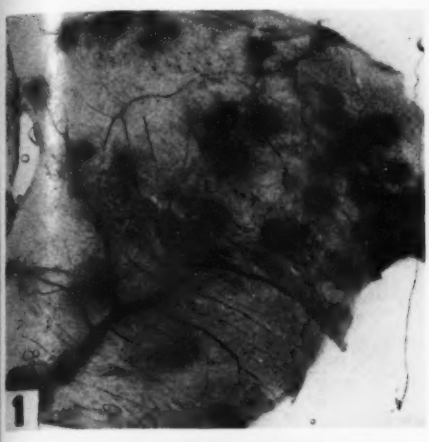
FIG. 6. Herpes. Same membrane as Figure 3. × 35.

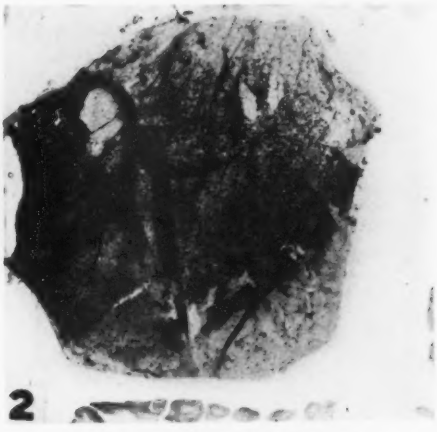
A

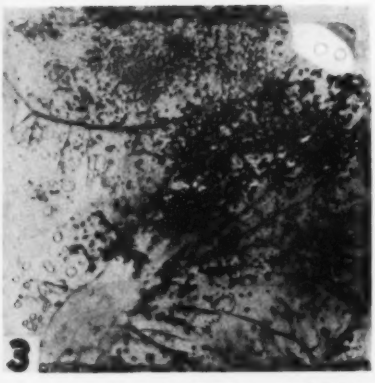
1

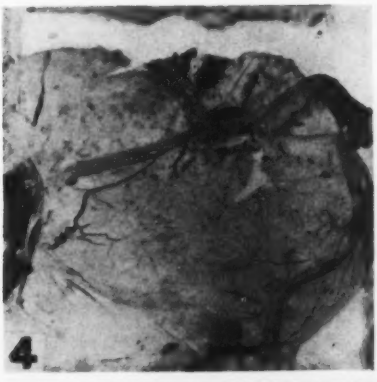
5

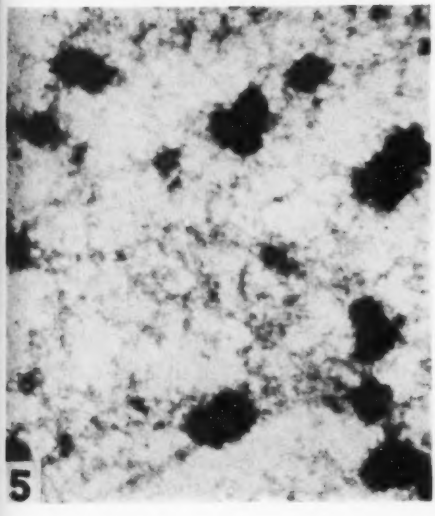
C

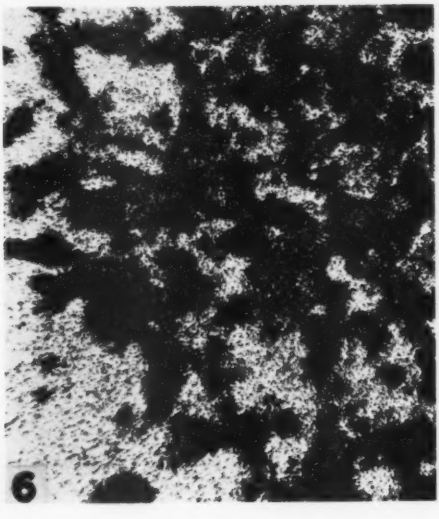












Cooke and Blattner

Trypan-Blue Staining of Chorio-allantoic Membranes

WARNING